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Introduction

class="introduction"

Most environments, including cars, are not sterile.

A study[[footnote](#)] analyzed 11 locations within 18 different cars to determine the number of microbial colony-forming units (CFUs) present. The center console harbored by far the most microbes (506 CFUs), possibly because that is where drinks are placed (and often spilled). Frequently touched sites also had high concentrations. (credit “photo”: modification

of work by Jeff
 Wilcox)
 R.E.
 Stephenson et
 al.
 “Elucidation of
 Bacteria Found
 in Car Interiors
 and Strategies
 to Reduce the
 Presence of
 Potential
 Pathogens.”
Biofouling 30
 no. 3
 (2014):337–
 346.

Location	Average number CFUs per 6.5 × 6.5 cm area
Door latch	256
Door lock	14
Door lock control	182
Door handle	29
Window control	4
Cruise control button	69
Steering wheel	239
Interior steering wheel	390
Radio volume knob	99
Gear shifter	115
Center console	506



How clean is clean? People wash their cars and vacuum the carpets, but most would not want to eat from these surfaces. Similarly, we might eat with silverware cleaned in a dishwasher, but we could not use the same dishwasher to clean surgical instruments. As these examples illustrate, “clean” is a relative term. Car washing, vacuuming, and dishwashing all reduce the microbial load on the items treated, thus making them “cleaner.” But whether they are “clean enough” depends on their intended use.

Because people do not normally eat from cars or carpets, these items do not require the same level of cleanliness that silverware does. Likewise, because silverware is not used for invasive surgery, these utensils do not require the same level of cleanliness as surgical equipment, which requires sterilization to prevent infection.

Why not play it safe and sterilize everything? Sterilizing everything we come in contact with is impractical, as well as potentially dangerous. As this chapter will demonstrate, sterilization protocols often require time- and labor-intensive treatments that may degrade the quality of the item being treated or have toxic effects on users. Therefore, the user must consider the item's intended application when choosing a cleaning method to ensure that it is "clean enough."

Controlling Microbial Growth

LEARNING OBJECTIVES

- Compare disinfectants, antiseptics, and sterilants
- Describe the principles of controlling the presence of microorganisms through sterilization and disinfection
- Differentiate between microorganisms of various biological safety levels and explain methods used for handling microbes at each level

Note:

Part 1

Roberta is a 46-year-old real estate agent who recently underwent a cholecystectomy (surgery to remove painful gallstones). The surgery was performed laparoscopically with the aid of a duodenoscope, a specialized endoscope that allows surgeons to see inside the body with the aid of a tiny camera. On returning home from the hospital, Roberta developed abdominal pain and a high fever. She also experienced a burning sensation during urination and noticed blood in her urine. She notified her surgeon of these symptoms, per her postoperative instructions.

- What are some possible causes of Roberta's symptoms?

Jump to the [next](#) Clinical Focus box.

To prevent the spread of human disease, it is necessary to control the growth and abundance of microbes in or on various items frequently used by humans. Inanimate items, such as doorknobs, toys, or towels, which may harbor microbes and aid in disease transmission, are called **fomites**. Two factors heavily influence the level of cleanliness required for a particular fomite and, hence, the protocol chosen to achieve this level. The first factor is the application for which the item will be used. For example, invasive applications that require insertion into the human body require a much higher level of cleanliness than applications that do not. The second factor is the level of resistance to antimicrobial treatment by potential pathogens. For example, foods preserved by canning often become contaminated with the bacterium *Clostridium botulinum*, which produces the neurotoxin that causes botulism. Because *C. botulinum* can produce endospores that can survive harsh conditions, extreme temperatures and pressures must be used to eliminate the endospores. Other organisms may not require such extreme measures and can be controlled by a procedure such as washing clothes in a laundry machine.

Laboratory Biological Safety Levels

For researchers or laboratory personnel working with pathogens, the risks associated with specific pathogens determine the levels of cleanliness and control required. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) have established four classification levels, called “biological safety levels” (BSLs). Various organizations around the world, including the World Health Organization (WHO) and the European Union (EU), use a similar classification scheme. According to the CDC, the BSL is determined by the agent’s infectivity, ease of transmission, and potential disease severity, as well as the type of work being done with the agent.[\[footnote\]](#)

US Centers for Disease Control and Prevention. “Recognizing the Biosafety Levels.” <http://www.cdc.gov/training/quicklearns/biosafety/>. Accessed June 7, 2016.

Each BSL requires a different level of biocontainment to prevent contamination and spread of infectious agents to laboratory personnel and, ultimately, the community. For example, the lowest BSL, BSL-1, requires

the fewest precautions because it applies to situations with the lowest risk for microbial infection.

BSL-1 agents are those that generally do not cause infection in healthy human adults. These include noninfectious bacteria, such as nonpathogenic strains of *Escherichia coli* and *Bacillus subtilis*, and viruses known to infect animals other than humans, such as baculoviruses (insect viruses). Because working with BSL-1 agents poses very little risk, few precautions are necessary. Laboratory workers use standard aseptic technique and may work with these agents at an open laboratory bench or table, wearing personal protective equipment (PPE) such as a laboratory coat, goggles, and gloves, as needed. Other than a sink for handwashing and doors to separate the laboratory from the rest of the building, no additional modifications are needed.

Agents classified as BSL-2 include those that pose moderate risk to laboratory workers and the community, and are typically “indigenous,” meaning that they are commonly found in that geographical area. These include bacteria such as *Staphylococcus aureus* and *Salmonella* spp., and viruses like hepatitis, mumps, and measles viruses. BSL-2 laboratories require additional precautions beyond those of BSL-1, including restricted access; required PPE, including a face shield in some circumstances; and the use of biological safety cabinets for procedures that may disperse agents through the air (called “aerosolization”). BSL-2 laboratories are equipped with self-closing doors, an eyewash station, and an **autoclave**, which is a specialized device for sterilizing materials with pressurized steam before use or disposal. BSL-1 laboratories may also have an autoclave.

BSL-3 agents have the potential to cause lethal infections by inhalation. These may be either indigenous or “exotic,” meaning that they are derived from a foreign location, and include pathogens such as *Mycobacterium tuberculosis*, *Bacillus anthracis*, West Nile virus, and human immunodeficiency virus (HIV). Because of the serious nature of the infections caused by BSL-3 agents, laboratories working with them require restricted access. Laboratory workers are under medical surveillance, possibly receiving vaccinations for the microbes with which they work. In addition to the standard PPE already mentioned, laboratory personnel in

BSL-3 laboratories must also wear a respirator and work with microbes and infectious agents in a biological safety cabinet at all times. BSL-3 laboratories require a hands-free sink, an eyewash station near the exit, and two sets of self-closing and locking doors at the entrance. These laboratories are equipped with directional airflow, meaning that clean air is pulled through the laboratory from clean areas to potentially contaminated areas. This air cannot be recirculated, so a constant supply of clean air is required.

BSL-4 agents are the most dangerous and often fatal. These microbes are typically exotic, are easily transmitted by inhalation, and cause infections for which there are no treatments or vaccinations. Examples include Ebola virus and Marburg virus, both of which cause hemorrhagic fevers, and smallpox virus. There are only a small number of laboratories in the United States and around the world appropriately equipped to work with these agents. In addition to BSL-3 precautions, laboratory workers in BSL-4 facilities must also change their clothing on entering the laboratory, shower on exiting, and decontaminate all material on exiting. While working in the laboratory, they must either wear a full-body protective suit with a designated air supply or conduct all work within a biological safety cabinet with a high-efficiency particulate air (HEPA)-filtered air supply and a doubly HEPA-filtered exhaust. If wearing a suit, the air pressure within the suit must be higher than that outside the suit, so that if a leak in the suit occurs, laboratory air that may be contaminated cannot be drawn into the suit ([\[link\]](#)). The laboratory itself must be located either in a separate building or in an isolated portion of a building and have its own air supply and exhaust system, as well as its own decontamination system. The BSLs are summarized in [\[link\]](#).



A protective suit like this one is an additional precaution for those who work in BSL-4 laboratories. This suit has its own air supply and maintains a positive pressure relative to the outside, so that if a leak occurs, air will flow out of the suit, not into it from the laboratory. (credit: modification of work by Centers for Disease Control and Prevention)

Biosafety Levels			
Biological Safety Levels	Description	Examples	CDC Classification
BSL-4	Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level.	Ebola and Marburg viruses	
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	<i>Mycobacterium tuberculosis</i>	
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	<i>Staphylococcus aureus</i>	
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of <i>Escherichia coli</i>	

The CDC classifies infectious agents into four biosafety levels based on potential risk to laboratory personnel and the community. Each level requires a progressively greater level of precaution. (credit “pyramid”: modification of work by Centers for Disease Control and Prevention)

Note:

To [learn more](#) about the four BSLs, visit the CDC’s website.

Note:

- What are some factors used to determine the BSL necessary for working with a specific pathogen?

Sterilization

The most extreme protocols for microbial control aim to achieve **sterilization**: the complete removal or killing of all vegetative cells, endospores, and viruses from the targeted item or environment. Sterilization protocols are generally reserved for laboratory, medical, manufacturing, and food industry settings, where it may be imperative for certain items to be completely free of potentially infectious agents. Sterilization can be accomplished through either physical means, such as exposure to high heat, pressure, or filtration through an appropriate filter, or by chemical means. Chemicals that can be used to achieve sterilization are called **sterilants**. Sterilants effectively kill all microbes and viruses, and, with appropriate exposure time, can also kill endospores.

For many clinical purposes, **aseptic technique** is necessary to prevent contamination of sterile surfaces. Aseptic technique involves a combination of protocols that collectively maintain sterility, or **asepsis**, thus preventing contamination of the patient with microbes and infectious agents. Failure to practice aseptic technique during many types of clinical procedures may introduce microbes to the patient's body and put the patient at risk for **sepsis**, a systemic inflammatory response to an infection that results in high fever, increased heart and respiratory rates, shock, and, possibly, death. Medical procedures that carry risk of contamination must be performed in a **sterile field**, a designated area that is kept free of all vegetative microbes, endospores, and viruses. Sterile fields are created according to protocols requiring the use of sterilized materials, such as packaging and drapings, and strict procedures for washing and application of sterilants. Other protocols are followed to maintain the sterile field while the medical procedure is being performed.

One food sterilization protocol, **commercial sterilization**, uses heat at a temperature low enough to preserve food quality but high enough to destroy common pathogens responsible for food poisoning, such as *C. botulinum*. Because *C. botulinum* and its endospores are commonly found in soil, they

may easily contaminate crops during harvesting, and these endospores can later germinate within the anaerobic environment once foods are canned. Metal cans of food contaminated with *C. botulinum* will bulge due to the microbe's production of gases; contaminated jars of food typically bulge at the metal lid. To eliminate the risk for *C. botulinum* contamination, commercial food-canning protocols are designed with a large margin of error. They assume an impossibly large population of endospores (10^{12} per can) and aim to reduce this population to 1 endospore per can to ensure the safety of canned foods. For example, low- and medium-acid foods are heated to 121 °C for a minimum of 2.52 minutes, which is the time it would take to reduce a population of 10^{12} endospores per can down to 1 endospore at this temperature. Even so, commercial sterilization does not eliminate the presence of all microbes; rather, it targets those pathogens that cause spoilage and foodborne diseases, while allowing many nonpathogenic organisms to survive. Therefore, “sterilization” is somewhat of a misnomer in this context, and commercial sterilization may be more accurately described as “quasi-sterilization.”

Note:

- What is the difference between sterilization and aseptic technique?

Note:

The Association of Surgical Technologists publishes [standards](#) for aseptic technique, including creating and maintaining a sterile field.

Other Methods of Control

Sterilization protocols require procedures that are not practical, or necessary, in many settings. Various other methods are used in clinical and nonclinical settings to reduce the microbial load on items. Although the

terms for these methods are often used interchangeably, there are important distinctions ([\[link\]](#)).

The process of **disinfection** inactivates most microbes on the surface of a fomite by using antimicrobial chemicals or heat. Because some microbes remain, the disinfected item is not considered sterile. Ideally, **disinfectants** should be fast acting, stable, easy to prepare, inexpensive, and easy to use. An example of a natural disinfectant is vinegar; its acidity kills most microbes. Chemical disinfectants, such as chlorine bleach or products containing chlorine, are used to clean nonliving surfaces such as laboratory benches, clinical surfaces, and bathroom sinks. Typical disinfection does not lead to sterilization because endospores tend to survive even when all vegetative cells have been killed.

Unlike disinfectants, **antiseptics** are antimicrobial chemicals safe for use on living skin or tissues. Examples of antiseptics include hydrogen peroxide and isopropyl alcohol. The process of applying an antiseptic is called **antiseptis**. In addition to the characteristics of a good disinfectant, antiseptics must also be selectively effective against microorganisms and able to penetrate tissue deeply without causing tissue damage.

The type of protocol required to achieve the desired level of cleanliness depends on the particular item to be cleaned. For example, those used clinically are categorized as critical, semicritical, and noncritical. Critical items must be sterile because they will be used inside the body, often penetrating sterile tissues or the bloodstream; examples of **critical items** include surgical instruments, catheters, and intravenous fluids. Gastrointestinal endoscopes and various types of equipment for respiratory therapies are examples of **semicritical items**; they may contact mucous membranes or nonintact skin but do not penetrate tissues. Semicritical items do not typically need to be sterilized but do require a high level of disinfection. Items that may contact but not penetrate intact skin are **noncritical items**; examples are bed linens, furniture, crutches, stethoscopes, and blood pressure cuffs. These articles need to be clean but not highly disinfected.

The act of handwashing is an example of **degerming**, in which microbial numbers are significantly reduced by gently scrubbing living tissue, most

commonly skin, with a mild chemical (e.g., soap) to avoid the transmission of pathogenic microbes. Wiping the skin with an alcohol swab at an injection site is another example of degerming. These degerming methods remove most (but not all) microbes from the skin's surface.

The term **sanitization** refers to the cleansing of fomites to remove enough microbes to achieve levels deemed safe for public health. For example, commercial dishwashers used in the food service industry typically use very hot water and air for washing and drying; the high temperatures kill most microbes, sanitizing the dishes. Surfaces in hospital rooms are commonly sanitized using a chemical disinfectant to prevent disease transmission between patients. [\[link\]](#) summarizes common protocols, definitions, applications, and agents used to control microbial growth.

Common Protocols for Control of Microbial Growth			
Protocol	Definition	Common Application	Common Agents
For Use on Fomites			
Disinfection	Reduces or destroys microbial load of an inanimate item through application of heat or antimicrobial chemicals	Cleaning surfaces like laboratory benches, clinical surfaces, and bathrooms	Chlorine bleach, phenols (e.g., Lysol), glutaraldehyde
Sanitization	Reduces microbial load of an inanimate item to safe public health levels through application of heat or antimicrobial chemicals	Commercial dishwashing of eating utensils, cleaning public restrooms	Detergents containing phosphates (e.g., Finish), industrial-strength cleaners containing quaternary ammonium compounds
Sterilization	Completely eliminates all vegetative cells, endospores, and viruses from an inanimate item	Preparation of surgical equipment and of needles used for injection	Pressurized steam (autoclave), chemicals, radiation
For Use on Living Tissue			
Antisepsis	Reduces microbial load on skin or tissue through application of an antimicrobial chemical	Cleaning skin broken due to injury; cleaning skin before surgery	Boric acid, isopropyl alcohol, hydrogen peroxide, iodine (betadine)
Degerming	Reduces microbial load on skin or tissue through gentle to firm scrubbing and the use of mild chemicals	Handwashing	Soap, alcohol swab

Note:

- What is the difference between a disinfectant and an antiseptic?
- Which is most effective at removing microbes from a product: sanitization, degerming, or sterilization? Explain.

Note:**Part 2**

Roberta's physician suspected that a bacterial infection was responsible for her sudden-onset high fever, abdominal pain, and bloody urine. Based on these symptoms, the physician diagnosed a urinary tract infection (UTI). A wide variety of bacteria may cause UTIs, which typically occur when bacteria from the lower gastrointestinal tract are introduced to the urinary tract. However, Roberta's recent gallstone surgery caused the physician to suspect that she had contracted a nosocomial (hospital-acquired) infection during her surgery. The physician took a urine sample and ordered a urine culture to check for the presence of white blood cells, red blood cells, and bacteria. The results of this test would help determine the cause of the infection. The physician also prescribed a course of the antibiotic ciprofloxacin, confident that it would clear Roberta's infection.

- What are some possible ways that bacteria could have been introduced to Roberta's urinary tract during her surgery?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

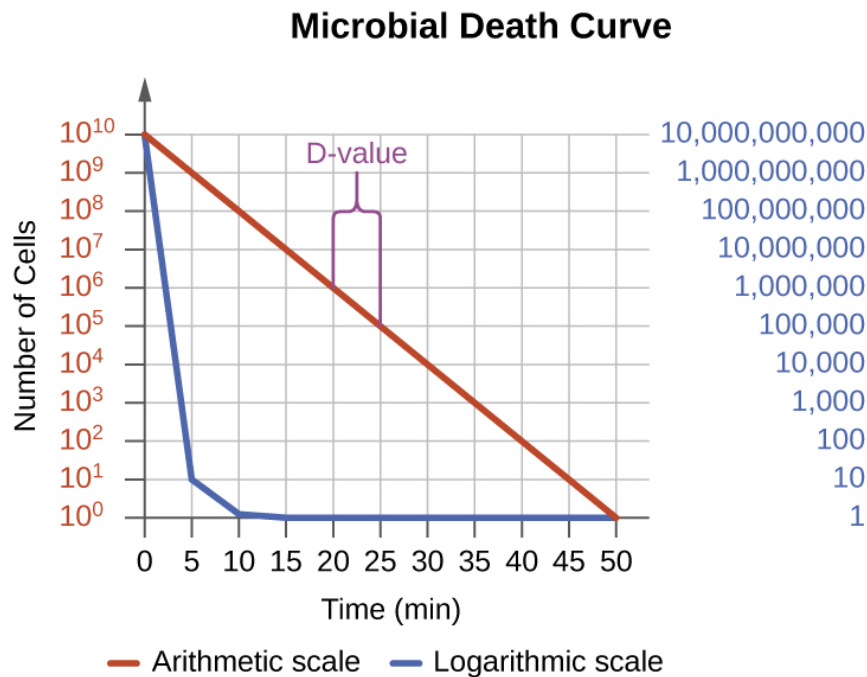
Measuring Microbial Control

Physical and chemical methods of microbial control that kill the targeted microorganism are identified by the suffix *-cide* (or *-cidal*). The prefix indicates the type of microbe or infectious agent killed by the treatment method: **bactericides** kill bacteria, **viricides** kill or inactivate viruses, and

fungicides kill fungi. Other methods do not kill organisms but, instead, stop their growth, making their population static; such methods are identified by the suffix *-stat* (or *-static*). For example, **bacteriostatic** treatments inhibit the growth of bacteria, whereas **fungistatic** treatments inhibit the growth of fungi. Factors that determine whether a particular treatment is *-cidal* or *-static* include the types of microorganisms targeted, the concentration of the chemical used, and the nature of the treatment applied.

Although *-static* treatments do not actually kill infectious agents, they are often less toxic to humans and other animals, and may also better preserve the integrity of the item treated. Such treatments are typically sufficient to keep the microbial population of an item in check. The reduced toxicity of some of these *-static* chemicals also allows them to be impregnated safely into plastics to prevent the growth of microbes on these surfaces. Such plastics are used in products such as toys for children and cutting boards for food preparation. When used to treat an infection, *-static* treatments are typically sufficient in an otherwise healthy individual, preventing the pathogen from multiplying, thus allowing the individual's immune system to clear the infection.

The degree of microbial control can be evaluated using a **microbial death curve** to describe the progress and effectiveness of a particular protocol. When exposed to a particular microbial control protocol, a fixed percentage of the microbes within the population will die. Because the rate of killing remains constant even when the population size varies, the percentage killed is more useful information than the absolute number of microbes killed. Death curves are often plotted as semilog plots just like microbial growth curves because the reduction in microorganisms is typically logarithmic ([\[link\]](#)). The amount of time it takes for a specific protocol to produce a one order-of-magnitude decrease in the number of organisms, or the death of 90% of the population, is called the **decimal reduction time (DRT)** or **D-value**.



Time (min)	Number of cells
0	10^{10}
5	10^9
10	10^8
15	10^7
20	10^6
25	10^5
30	10^4
35	10^3
40	10^2
45	10^1
50	10^0

Microbial death is logarithmic and easily observed using a semilog plot instead of an arithmetic one. The decimal reduction time (D-value) is the time it takes to kill 90% of the population (a 1-log decrease in the total population) when exposed to a specific microbial control protocol, as indicated by the purple bracket.

Several factors contribute to the effectiveness of a disinfecting agent or microbial control protocol. First, as demonstrated in [\[link\]](#), the length of time of exposure is important. Longer exposure times kill more microbes. Because microbial death of a population exposed to a specific protocol is logarithmic, it takes longer to kill a high-population load than a low-population load exposed to the same protocol. A shorter treatment time (measured in multiples of the D-value) is needed when starting with a smaller number of organisms. Effectiveness also depends on the susceptibility of the agent to that disinfecting agent or protocol. The concentration of disinfecting agent or intensity of exposure is also important. For example, higher temperatures and higher concentrations of

disinfectants kill microbes more quickly and effectively. Conditions that limit contact between the agent and the targeted cells—for example, the presence of bodily fluids, tissue, organic debris (e.g., mud or feces), or biofilms on surfaces—increase the cleaning time or intensity of the microbial control protocol required to reach the desired level of cleanliness. All these factors must be considered when choosing the appropriate protocol to control microbial growth in a given situation.

Note:

- What are two possible reasons for choosing a bacteriostatic treatment over a bactericidal one?
- Name at least two factors that can compromise the effectiveness of a disinfecting agent.

Key Concepts and Summary

- Inanimate items that may harbor microbes and aid in their transmission are called **fomites**. The level of cleanliness required for a fomite depends both on the item's use and the infectious agent with which the item may be contaminated.
- The CDC and the NIH have established four **biological safety levels (BSLs)** for laboratories performing research on infectious agents. Each level is designed to protect laboratory personnel and the community. These BSLs are determined by the agent's infectivity, ease of transmission, and potential disease severity, as well as the type of work being performed with the agent.
- **Disinfection** removes potential pathogens from a fomite, whereas **antisepsis** uses antimicrobial chemicals safe enough for tissues; in both cases, microbial load is reduced, but microbes may remain unless the chemical used is strong enough to be a **sterilant**.
- The amount of cleanliness (**sterilization** versus high-level disinfection versus general cleanliness) required for items used clinically depends

on whether the item will come into contact with sterile tissues (**critical item**), mucous membranes (**semicritical item**), or intact skin (**noncritical item**).

- Medical procedures with a risk for contamination should be carried out in a **sterile field** maintained by proper **aseptic technique** to prevent **sepsis**.
- Sterilization is necessary for some medical applications as well as in the food industry, where endospores of *Clostridium botulinum* are killed through **commercial sterilization** protocols.
- Physical or chemical methods to control microbial growth that result in death of the microbe are indicated by the suffixes *-cide* or *-cidal* (e.g., as with **bactericides**, **viricides**, and **fungicides**), whereas those that inhibit microbial growth are indicated by the suffixes *-stat* or *-static* (e.g., **bacteriostatic**, **fungistatic**).
- **Microbial death curves** display the logarithmic decline of living microbes exposed to a method of microbial control. The time it takes for a protocol to yield a 1-log (90%) reduction in the microbial population is the **decimal reduction time**, or **D-value**.
- When choosing a microbial control protocol, factors to consider include the length of exposure time, the type of microbe targeted, its susceptibility to the protocol, the intensity of the treatment, the presence of organics that may interfere with the protocol, and the environmental conditions that may alter the effectiveness of the protocol.

Multiple Choice

Exercise:

Problem:

Which of the following types of medical items requires sterilization?

- A. needles
- B. bed linens
- C. respiratory masks
- D. blood pressure cuffs

Solution:

A

Exercise:

Problem:

Which of the following is suitable for use on tissues for microbial control to prevent infection?

- A. disinfectant
- B. antiseptic
- C. sterilant
- D. water

Solution:

B

Exercise:

Problem:

Which biosafety level is appropriate for research with microbes or infectious agents that pose moderate risk to laboratory workers and the community, and are typically indigenous?

- A. BSL-1
- B. BSL-2
- C. BSL-3
- D. BSL-4

Solution:

B

Exercise:

Problem:

Which of the following best describes a microbial control protocol that inhibits the growth of molds and yeast?

- A. bacteriostatic
- B. fungicidal
- C. bactericidal
- D. fungistatic

Solution:

D

Exercise:**Problem:**

The decimal reduction time refers to the amount of time it takes to which of the following?

- A. reduce a microbial population by 10%
- B. reduce a microbial population by 0.1%
- C. reduce a microbial population by 90%
- D. completely eliminate a microbial population

Solution:

C

Fill in the Blank**Exercise:**

Problem:

A medical item that comes into contact with intact skin and does not penetrate sterile tissues or come into contact with mucous membranes is called a(n) _____ item.

Solution:

noncritical

Exercise:**Problem:**

The goal of _____ protocols is to rid canned produce of *Clostridium botulinum* endospores.

Solution:

commercial sterilization

True/False**Exercise:**

Problem: Sanitization leaves an object free of microbes.

Solution:

False

Short Answer**Exercise:**

Problem:

What are some characteristics of microbes and infectious agents that would require handling in a BSL-3 laboratory?

Exercise:**Problem:**

What is the purpose of degerming? Does it completely eliminate microbes?

Exercise:**Problem:**

What are some factors that alter the effectiveness of a disinfectant?

Critical Thinking**Exercise:****Problem:**

When plotting microbial death curves, how might they look different for bactericidal versus bacteriostatic treatments?

Exercise:**Problem:**

What are the benefits of cleaning something to a level of cleanliness beyond what is required? What are some possible disadvantages of doing so?

Using Physical Methods to Control Microorganisms

LEARNING OBJECTIVES

- Understand and compare various physical methods of controlling microbial growth, including heating, refrigeration, freezing, high-pressure treatment, desiccation, lyophilization, irradiation, and filtration

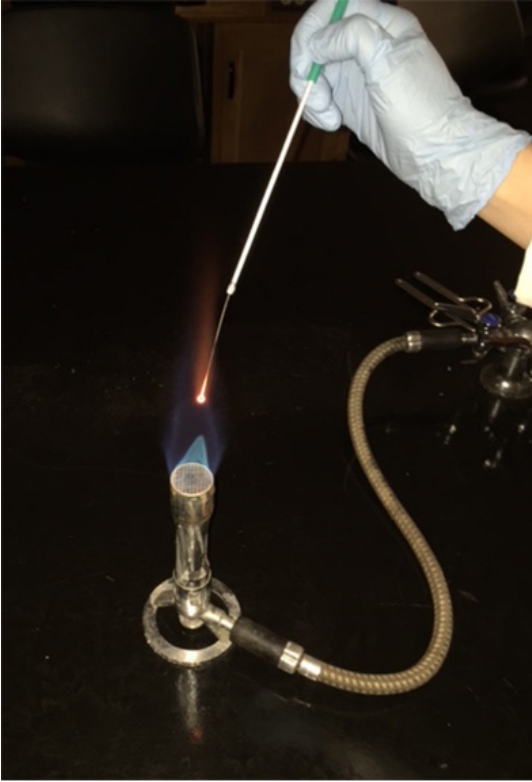
For thousands of years, humans have used various physical methods of microbial control for food preservation. Common control methods include the application of high temperatures, radiation, filtration, and desiccation (drying), among others. Many of these methods nonspecifically kill cells by disrupting membranes, changing membrane permeability, or damaging proteins and nucleic acids by denaturation, degradation, or chemical modification. Various physical methods used for microbial control are described in this section.

Heat

Heating is one of the most common—and oldest—forms of microbial control. It is used in simple techniques like cooking and canning. Heat can kill microbes by altering their membranes and denaturing proteins. The **thermal death point (TDP)** of a microorganism is the lowest temperature at which all microbes are killed in a 10-minute exposure. Different microorganisms will respond differently to high temperatures, with some (e.g., endospore-formers such as *C. botulinum*) being more heat tolerant. A similar parameter, the **thermal death time (TDT)**, is the length of time needed to kill all microorganisms in a sample at a given temperature. These parameters are often used to describe sterilization procedures that use high heat, such as autoclaving. Boiling is one of the oldest methods of moist-heat control of

microbes, and it is typically quite effective at killing vegetative cells and some viruses. However, boiling is less effective at killing endospores; some endospores are able to survive up to 20 hours of boiling. Additionally, boiling may be less effective at higher altitudes, where the boiling point of water is lower and the boiling time needed to kill microbes is therefore longer. For these reasons, boiling is not considered a useful sterilization technique in the laboratory or clinical setting.

Many different heating protocols can be used for sterilization in the laboratory or clinic, and these protocols can be broken down into two main categories: **dry-heat sterilization** and **moist-heat sterilization**. Aseptic technique in the laboratory typically involves some dry-heat sterilization protocols using direct application of high heat, such as sterilizing inoculating loops ([link](#)). Incineration at very high temperatures destroys all microorganisms. Dry heat can also be applied for relatively long periods of time (at least 2 hours) at temperatures up to 170 °C by using a dry-heat sterilizer, such as an oven. However, moist-heat sterilization is typically the more effective protocol because it penetrates cells better than dry heat does.



(a)



(b)

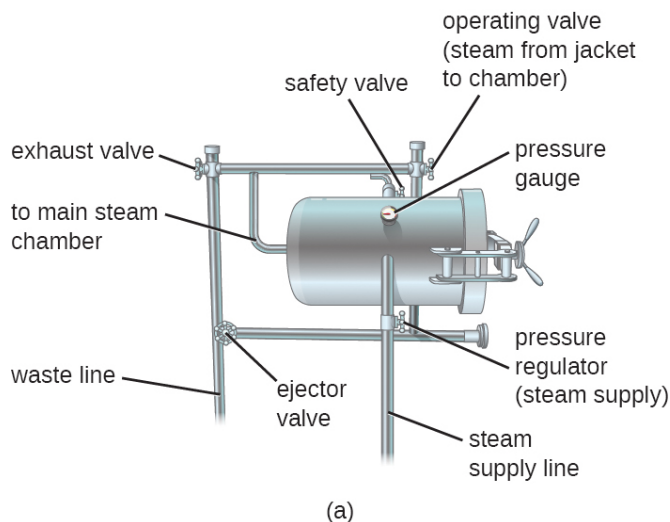
(a) Sterilizing a loop, often referred to as “flaming a loop,” is a common component of aseptic technique in the microbiology laboratory and is used to incinerate any microorganisms on the loop. (b) Alternatively, a bactericinerator may be used to reduce aerosolization of microbes and remove the presence of an open flame in the laboratory. These are examples of dry-heat sterilization by the direct application of high heat capable of incineration. (credit a: modification of work by Anh-Hue Tu; credit b: modification of work by Brian Forster)

Autoclaves

Autoclaves rely on moist-heat sterilization. They are used to raise temperatures above the boiling point of water to sterilize items such as surgical equipment from vegetative cells, viruses, and especially endospores, which are known to survive boiling temperatures, without damaging the items. Charles

Chamberland (1851–1908) designed the modern autoclave in 1879 while working in the laboratory of Louis Pasteur. The autoclave is still considered the most effective method of sterilization ([link](#)). Outside laboratory and clinical settings, large industrial autoclaves called **retorts** allow for moist-heat sterilization on a large scale.

In general, the air in the chamber of an autoclave is removed and replaced with increasing amounts of steam trapped within the enclosed chamber, resulting in increased interior pressure and temperatures above the boiling point of water. The two main types of autoclaves differ in the way that air is removed from the chamber. In gravity displacement autoclaves, steam is introduced into the chamber from the top or sides. Air, which is heavier than steam, sinks to the bottom of the chamber, where it is forced out through a vent. Complete displacement of air is difficult, especially in larger loads, so longer cycles may be required for such loads. In prevacuum sterilizers, air is removed completely using a high-speed vacuum before introducing steam into the chamber. Because air is more completely eliminated, the steam can more easily penetrate wrapped items. Many autoclaves are capable of both gravity and prevacuum cycles, using the former for the decontamination of waste and sterilization of media and unwrapped glassware, and the latter for sterilization of packaged instruments.



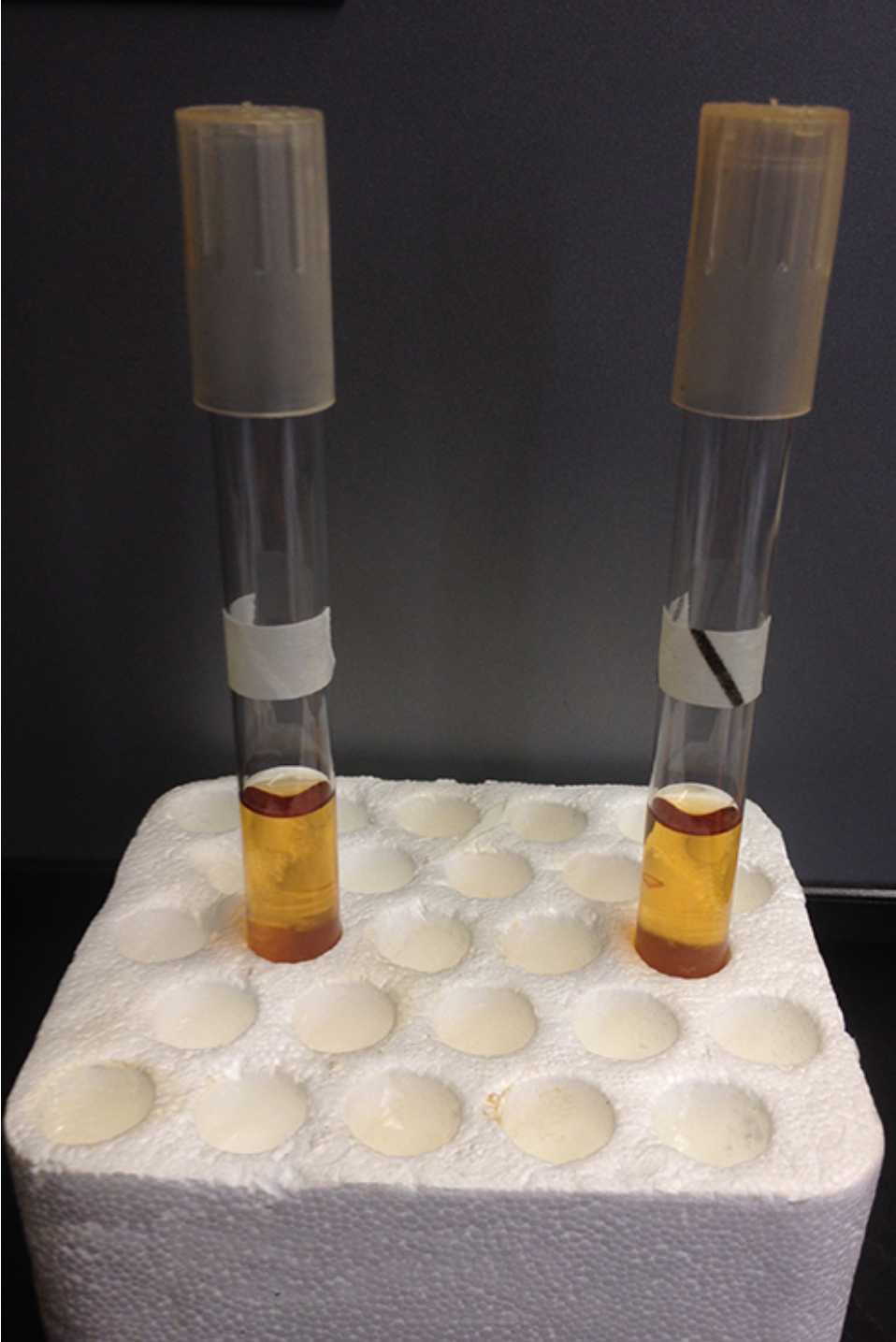
(a) An autoclave is commonly used for sterilization in the laboratory and in clinical settings. By displacing the air in the chamber with increasing

amounts of steam, pressure increases, and temperatures exceeding 100 °C can be achieved, allowing for complete sterilization. (b) A researcher programs an autoclave to sterilize a sample. (credit a: modification of work by Courtney Harrington; credit b: modification of work by Lackemeyer MG, Kok-Mercado Fd, Wada J, Bollinger L, Kindrachuk J, Wahl-Jensen V, Kuhn JH, Jahrling PB)

Standard operating temperatures for autoclaves are 121 °C or, in some cases, 132 °C, typically at a pressure of 15 to 20 pounds per square inch (psi). The length of exposure depends on the volume and nature of material being sterilized, but it is typically 20 minutes or more, with larger volumes requiring longer exposure times to ensure sufficient heat transfer to the materials being sterilized. The steam must directly contact the liquids or dry materials being sterilized, so containers are left loosely closed and instruments are loosely wrapped in paper or foil. The key to autoclaving is that the temperature must be high enough to kill endospores to achieve complete sterilization.

Because sterilization is so important to safe medical and laboratory protocols, quality control is essential. Autoclaves may be equipped with recorders to document the pressures and temperatures achieved during each run. Additionally, internal indicators of various types should be autoclaved along with the materials to be sterilized to ensure that the proper sterilization temperature has been reached ([link](#)). One common type of indicator is the use of heat-sensitive autoclave tape, which has white stripes that turn black when the appropriate temperature is achieved during a successful autoclave run. This type of indicator is relatively inexpensive and can be used during every run. However, autoclave tape provides no indication of length of exposure, so it cannot be used as an indicator of sterility. Another type of indicator, a biological indicator spore test, uses either a strip of paper or a liquid suspension of the endospores of *Geobacillus stearothermophilus* to determine whether the endospores are killed by the process. The endospores of the obligate thermophilic bacterium *G. stearothermophilus* are the gold standard used for this purpose because of their extreme heat resistance. Biological spore indicators can also be used to test the effectiveness of other sterilization protocols, including ethylene oxide, dry heat, formaldehyde, gamma radiation, and hydrogen peroxide plasma sterilization using either *G. stearothermophilus*, *Bacillus atrophaeus*, *B. subtilis*, or *B. pumilus* spores. In

the case of validating autoclave function, the endospores are incubated after autoclaving to ensure no viable endospores remain. Bacterial growth subsequent to endospore germination can be monitored by biological indicator spore tests that detect acid metabolites or fluorescence produced by enzymes derived from viable *G. stearothermophilus*. A third type of autoclave indicator is the Diack tube, a glass ampule containing a temperature-sensitive pellet that melts at the proper sterilization temperature. Spore strips or Diack tubes are used periodically to ensure the autoclave is functioning properly.

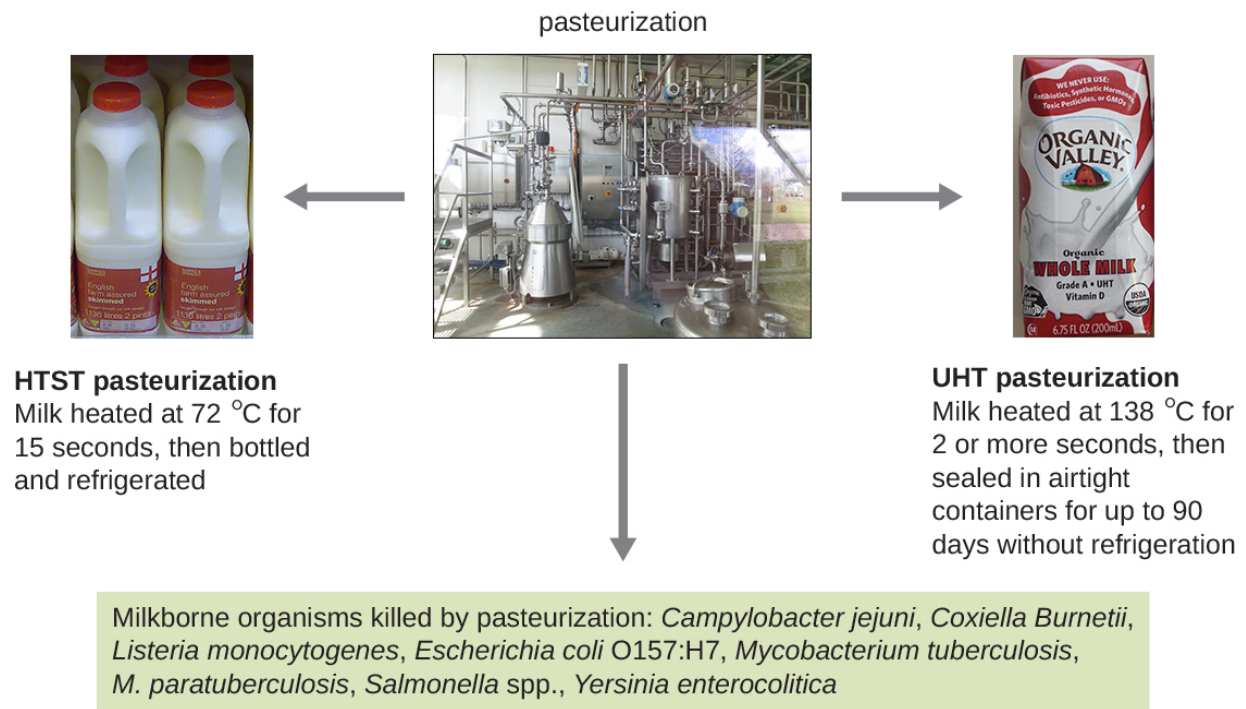


The white strips on autoclave tape (left tube) turn dark during a successful autoclave run (right tube). (credit: modification of work by Brian Forster)

Pasteurization

Although complete sterilization is ideal for many medical applications, it is not always practical for other applications and may also alter the quality of the product. Boiling and autoclaving are not ideal ways to control microbial growth in many foods because these methods may ruin the consistency and other organoleptic (sensory) qualities of the food. Pasteurization is a form of microbial control for food that uses heat but does not render the food sterile. Traditional **pasteurization** kills pathogens and reduces the number of spoilage-causing microbes while maintaining food quality. The process of pasteurization was first developed by Louis Pasteur in the 1860s as a method for preventing the spoilage of beer and wine. Today, pasteurization is most commonly used to kill heat-sensitive pathogens in milk and other food products (e.g., apple juice and honey) ([link](#)). However, because pasteurized food products are not sterile, they will eventually spoil.

The methods used for milk pasteurization balance the temperature and the length of time of treatment. One method, **high-temperature short-time (HTST) pasteurization**, exposes milk to a temperature of 72 °C for 15 seconds, which lowers bacterial numbers while preserving the quality of the milk. An alternative is **ultra-high-temperature (UHT) pasteurization**, in which the milk is exposed to a temperature of 138 °C for 2 or more seconds. UHT pasteurized milk can be stored for a long time in sealed containers without being refrigerated; however, the very high temperatures alter the proteins in the milk, causing slight changes in the taste and smell. Still, this method of pasteurization is advantageous in regions where access to refrigeration is limited.



Two different methods of pasteurization, HTST and UHT, are commonly used to kill pathogens associated with milk spoilage. (credit left: modification of work by Mark Hillary; credit right: modification of work by Kerry Ceszyk)

Note:

- In an autoclave, how are temperatures above boiling achieved?
- How would the onset of spoilage compare between HTST-pasteurized and UHT-pasteurized milk?
- Why is boiling not used as a sterilization method in a clinical setting?

Refrigeration and Freezing

Just as high temperatures are effective for controlling microbial growth, exposing microbes to low temperatures can also be an easy and effective method of microbial control, with the exception of psychrophiles, which prefer cold temperatures (see [Temperature and Microbial Growth](#)). Refrigerators used in home kitchens or in the laboratory maintain temperatures between 0 °C and 7 °C. This temperature range inhibits microbial metabolism, slowing the growth of microorganisms significantly and helping preserve refrigerated products such as foods or medical supplies. Certain types of laboratory cultures can be preserved by refrigeration for later use.

Freezing below –2 °C may stop microbial growth and even kill susceptible organisms. According to the US Department of Agriculture (USDA), the only safe ways that frozen foods can be thawed are in the refrigerator, immersed in cold water changed every 30 minutes, or in the microwave, keeping the food at temperatures not conducive for bacterial growth. [\[footnote\]](#) In addition, halted bacterial growth can restart in thawed foods, so thawed foods should be treated like fresh perishables.

US Department of Agriculture. “Freezing and Food Safety.” 2013.
http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/freezing-and-food-safety/CT_Index. Accessed June 8, 2016.

Bacterial cultures and medical specimens requiring long-term storage or transport are often frozen at ultra-low temperatures of –70 °C or lower. These ultra-low temperatures can be achieved by storing specimens on dry ice in an ultra-low freezer or in special liquid nitrogen tanks, which maintain temperatures lower than –196 °C ([\[link\]](#)).



(a)



(b)

Cultures and other medical specimens can be stored for long periods at ultra-low temperatures. (a) An ultra-low freezer maintains temperatures at or below -70°C . (b) Even lower temperatures can be achieved through freezing and storage in liquid nitrogen. (credit a: modification of work by “Expert Infantry”/Flickr; credit b: modification of work by USDA)

Note:

- Does placing food in a refrigerator kill bacteria on the food?

Pressure

Exposure to high pressure kills many microbes. In the food industry, high-pressure processing (also called pascalization) is used to kill bacteria, yeast, molds, parasites, and viruses in foods while maintaining food quality and extending shelf life. The application of high pressure between 100 and 800

MPa (sea level atmospheric pressure is about 0.1 MPa) is sufficient to kill vegetative cells by protein denaturation, but endospores may survive these pressures. [\[footnote\]](#)[\[footnote\]](#)

C. Ferstl. “High Pressure Processing: Insights on Technology and Regulatory Requirements.” Food for Thought/White Paper. Series Volume 10. Livermore, CA: The National Food Lab; July 2013.

US Food and Drug Administration. “Kinetics of Microbial Inactivation for Alternative Food Processing Technologies: High Pressure Processing.” 2000. <http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm101456.htm>. Accessed July 19, 2106.

In clinical settings, hyperbaric oxygen therapy is sometimes used to treat infections. In this form of therapy, a patient breathes pure oxygen at a pressure higher than normal atmospheric pressure, typically between 1 and 3 atmospheres (atm). This is achieved by placing the patient in a hyperbaric chamber or by supplying the pressurized oxygen through a breathing tube. Hyperbaric oxygen therapy helps increase oxygen saturation in tissues that become hypoxic due to infection and inflammation. This increased oxygen concentration enhances the body’s immune response by increasing the activities of neutrophils and macrophages, white blood cells that fight infections. Increased oxygen levels also contribute to the formation of toxic free radicals that inhibit the growth of oxygen-sensitive or anaerobic bacteria like as *Clostridium perfringens*, a common cause of gas gangrene. In *C. perfringens* infections, hyperbaric oxygen therapy can also reduce secretion of a bacterial toxin that causes tissue destruction. Hyperbaric oxygen therapy also seems to enhance the effectiveness of antibiotic treatments. Unfortunately, some rare risks include oxygen toxicity and effects on delicate tissues, such as the eyes, middle ear, and lungs, which may be damaged by the increased air pressure.

High pressure processing is not commonly used for disinfection or sterilization of fomites. Although the application of pressure and steam in an autoclave is effective for killing endospores, it is the high temperature achieved, and not the pressure directly, that results in endospore death.

Note:

A Streak of Bad Potluck

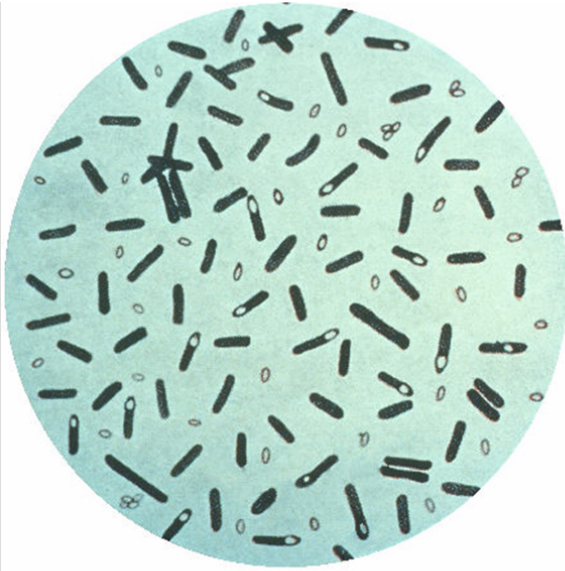
One Monday in spring 2015, an Ohio woman began to experience blurred, double vision; difficulty swallowing; and drooping eyelids. She was rushed to the emergency department of her local hospital. During the examination, she began to experience abdominal cramping, nausea, paralysis, dry mouth, weakness of facial muscles, and difficulty speaking and breathing. Based on these symptoms, the hospital's incident command center was activated, and Ohio public health officials were notified of a possible case of botulism. Meanwhile, other patients with similar symptoms began showing up at other local hospitals. Because of the suspicion of botulism, antitoxin was shipped overnight from the CDC to these medical facilities, to be administered to the affected patients. The first patient died of respiratory failure as a result of paralysis, and about half of the remaining victims required additional hospitalization following antitoxin administration, with at least two requiring ventilators for breathing.

Public health officials investigated each of the cases and determined that all of the patients had attended the same church potluck the day before. Moreover, they traced the source of the outbreak to a potato salad made with home-canned potatoes. More than likely, the potatoes were canned using boiling water, a method that allows endospores of *Clostridium botulinum* to survive. *C. botulinum* produces botulinum toxin, a neurotoxin that is often deadly once ingested. According to the CDC, the Ohio case was the largest botulism outbreak in the United States in nearly 40 years.[\[footnote\]](#)

CL McCarty et al. "Large Outbreak of Botulism Associated with a Church Potluck Meal-Ohio, 2015." *Morbidity and Mortality Weekly Report* 64, no. 29 (2015):802–803.

Killing *C. botulinum* endospores requires a minimum temperature of 116 °C (240 °F), well above the boiling point of water. This temperature can only be reached in a pressure canner, which is recommended for home canning of low-acid foods such as meat, fish, poultry, and vegetables ([\[link\]](#)).

Additionally, the CDC recommends boiling home-canned foods for about 10 minutes before consumption. Since the botulinum toxin is heat labile (meaning that it is denatured by heat), 10 minutes of boiling will render nonfunctional any botulinum toxin that the food may contain.



(a)



(b)

(a) *Clostridium botulinum* is the causative agent of botulism. (b) A pressure canner is recommended for home canning because endospores of *C. botulinum* can survive temperatures above the boiling point of water. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by National Center for Home Food Preservation)

Note:

To [learn more](#) about proper home-canning techniques, visit the CDC's website.

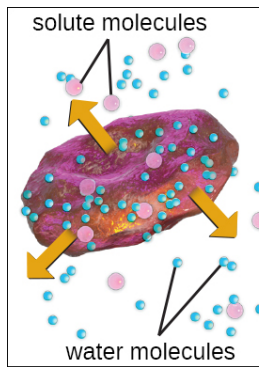
Desiccation

Drying, also known as **desiccation** or dehydration, is a method that has been used for millennia to preserve foods such as raisins, prunes, and jerky. It works because all cells, including microbes, require water for their metabolism and survival. Although drying controls microbial growth, it might not kill all

microbes or their endospores, which may start to regrow when conditions are more favorable and water content is restored.

In some cases, foods are dried in the sun, relying on evaporation to achieve desiccation. Freeze-drying, or **lyophilization**, is another method of dessication in which an item is rapidly frozen (“snap-frozen”) and placed under vacuum so that water is lost by sublimation. Lyophilization combines both exposure to cold temperatures and desiccation, making it quite effective for controlling microbial growth. In addition, lyophilization causes less damage to an item than conventional desiccation and better preserves the item’s original qualities. Lyophilized items may be stored at room temperature if packaged appropriately to prevent moisture acquisition. Lyophilization is used for preservation in the food industry and is also used in the laboratory for the long-term storage and transportation of microbial cultures.

The water content of foods and materials, called the **water activity**, can be lowered without physical drying by the addition of solutes such as salts or sugars. At very high concentrations of salts or sugars, the amount of available water in microbial cells is reduced dramatically because water will be drawn from an area of low solute concentration (inside the cell) to an area of high solute concentration (outside the cell) ([link](#)). Many microorganisms do not survive these conditions of high osmotic pressure. Honey, for example, is 80% sucrose, an environment in which very few microorganisms are capable of growing, thereby eliminating the need for refrigeration. Salted meats and fish, like ham and cod, respectively, were critically important foods before the age of refrigeration. Fruits were preserved by adding sugar, making jams and jellies. However, certain microbes, such as molds and yeasts, tend to be more tolerant of desiccation and high osmotic pressures, and, thus, may still contaminate these types of foods.



(a)



(b)

(a) The addition of a solute creates a hypertonic environment, drawing water out of cells. (b) Some foods can be dried directly, like raisins and jerky. Other foods are dried with the addition of salt, as in the case of salted fish, or sugar, as in the case of jam. (credit a: modification of work by “Bruce Blaus”/Wikimedia Commons; credit raisins: modification of work by Christian Schnettelker; credit jerky: modification of work by Larry Jacobsen; credit salted fish: modification of work by “The Photographer”/Wikimedia Commons; credit jam: modification of work by Kim Becker)

Note:

- How does the addition of salt or sugar to food affect its water activity?

Radiation

Radiation in various forms, from high-energy radiation to sunlight, can be used to kill microbes or inhibit their growth. **Ionizing radiation** includes X-rays, gamma rays, and high-energy electron beams. Ionizing radiation is strong enough to pass into the cell, where it alters molecular structures and damages cell components. For example, ionizing radiation introduces double-strand breaks in DNA molecules. This may directly cause DNA mutations to occur,

or mutations may be introduced when the cell attempts to repair the DNA damage. As these mutations accumulate, they eventually lead to cell death.

Both X-rays and gamma rays easily penetrate paper and plastic and can therefore be used to sterilize many packaged materials. In the laboratory, ionizing radiation is commonly used to sterilize materials that cannot be autoclaved, such as plastic Petri dishes and disposable plastic inoculating loops. For clinical use, ionizing radiation is used to sterilize gloves, intravenous tubing, and other latex and plastic items used for patient care. Ionizing radiation is also used for the sterilization of other types of delicate, heat-sensitive materials used clinically, including tissues for transplantation, pharmaceutical drugs, and medical equipment.

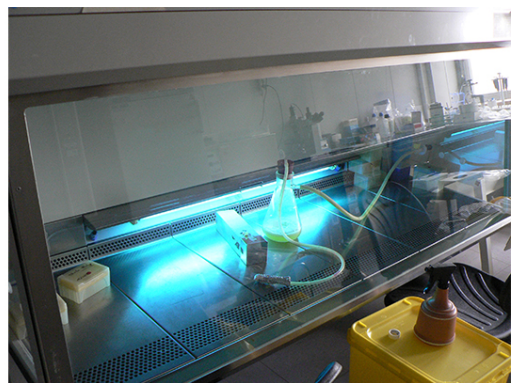
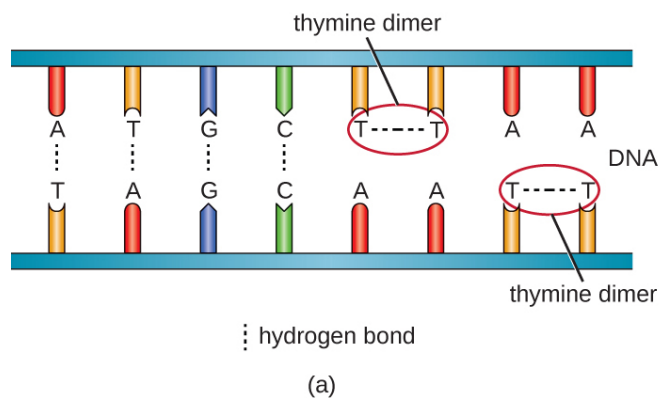
In Europe, gamma irradiation for food preservation is widely used, although it has been slow to catch on in the United States (see the [Micro Connections](#) box on this topic). Packaged dried spices are also often gamma-irradiated. Because of their ability to penetrate paper, plastic, thin sheets of wood and metal, and tissue, great care must be taken when using X-rays and gamma irradiation. These types of ionizing irradiation cannot penetrate thick layers of iron or lead, so these metals are commonly used to protect humans who may be potentially exposed.

Another type of radiation, **nonionizing radiation**, is commonly used for disinfection and uses less energy than ionizing radiation. It does not penetrate cells or packaging. Ultraviolet (UV) light is one example; it causes thymine dimers to form between adjacent thymines within a single strand of DNA ([link](#)). When DNA polymerase encounters the thymine dimer, it does not always incorporate the appropriate complementary nucleotides (two adenines), and this leads to formation of mutations that can ultimately kill microorganisms.

UV light can be used effectively by both consumers and laboratory personnel to control microbial growth. UV lamps are now commonly incorporated into water purification systems for use in homes. In addition, small portable UV lights are commonly used by campers to purify water from natural environments before drinking. Germicidal lamps are also used in surgical suites, biological safety cabinets, and transfer hoods, typically emitting UV light at a wavelength of 260 nm. Because UV light does not penetrate surfaces

and will not pass through plastics or glass, cells must be exposed directly to the light source.

Sunlight has a very broad spectrum that includes UV and visible light. In some cases, sunlight can be effective against certain bacteria because of both the formation of thymine dimers by UV light and by the production of reactive oxygen products induced in low amounts by exposure to visible light.



(a) UV radiation causes the formation of thymine dimers in DNA, leading to lethal mutations in the exposed microbes. (b) Germicidal lamps that emit UV light are commonly used in the laboratory to disinfect equipment.

Note:

- What are two advantages of ionizing radiation as a sterilization method?
- How does the effectiveness of ionizing radiation compare with that of nonionizing radiation?

Note:

Irradiated Food: Would You Eat That?

Of all the ways to prevent food spoilage and foodborne illness, gamma irradiation may be the most unappetizing. Although gamma irradiation is a proven method of eliminating potentially harmful microbes from food, the public has yet to buy in. Most of their concerns, however, stem from misinformation and a poor understanding of the basic principles of radiation. The most common method of irradiation is to expose food to cobalt-60 or cesium-137 by passing it through a radiation chamber on a conveyor belt. The food does not directly contact the radioactive material and does not become radioactive itself. Thus, there is no risk for exposure to radioactive material through eating gamma-irradiated foods. Additionally, irradiated foods are not significantly altered in terms of nutritional quality, aside from the loss of certain vitamins, which is also exacerbated by extended storage. Alterations in taste or smell may occur in irradiated foods with high fat content, such as fatty meats and dairy products, but this effect can be minimized by using lower doses of radiation at colder temperatures.

In the United States, the CDC, Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA) have deemed irradiation safe and effective for various types of meats, poultry, shellfish, fresh fruits and vegetables, eggs with shells, and spices and seasonings. Gamma irradiation of foods has also been approved for use in many other countries, including France, the Netherlands, Portugal, Israel, Russia, China, Thailand, Belgium, Australia, and South Africa. To help ameliorate consumer concern and assist with education efforts, irradiated foods are now clearly labeled and marked with the international irradiation symbol, called the “radura” ([\[link\]](#)).

Consumer acceptance seems to be rising, as indicated by several recent studies.[\[footnote\]](#)

AM Johnson et al. “Consumer Acceptance of Electron-Beam Irradiated Ready-to-Eat Poultry Meats.” *Food Processing Preservation*, 28 no. 4 (2004):302–319.



(a)



(b)

(a) Foods are exposed to gamma radiation by passage on a conveyor belt through a radiation chamber. (b) Gamma-irradiated foods must be clearly labeled and display the irradiation symbol, known as the “radura.”
(credit a, b: modification of work by U.S. Department of Agriculture)

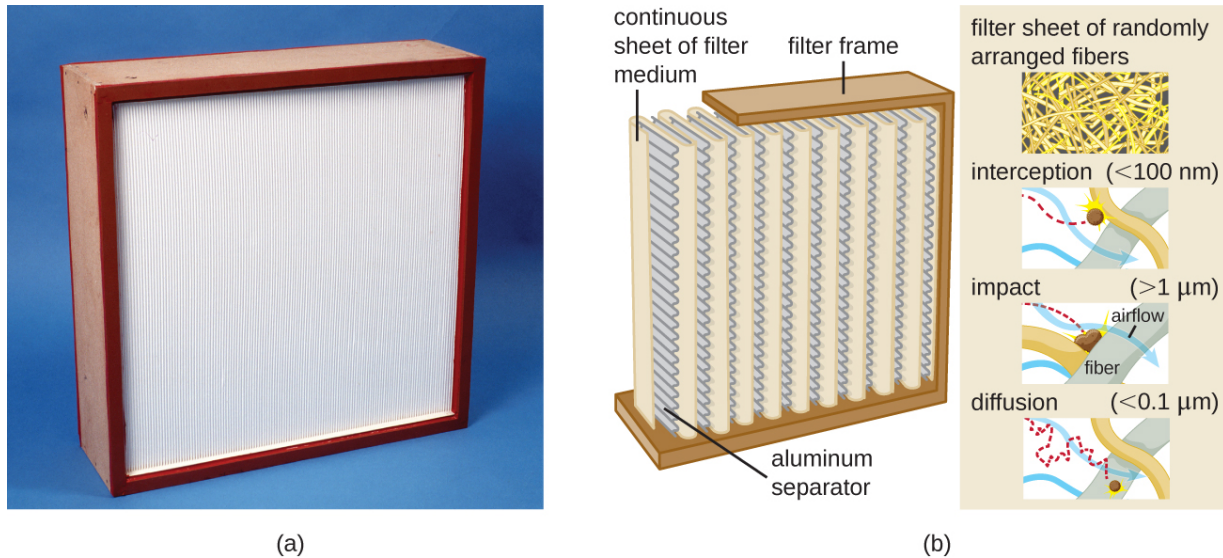
Sonication

The use of high-frequency ultrasound waves to disrupt cell structures is called **sonication**. Application of ultrasound waves causes rapid changes in pressure within the intracellular liquid; this leads to cavitation, the formation of bubbles inside the cell, which can disrupt cell structures and eventually cause the cell to lyse or collapse. Sonication is useful in the laboratory for efficiently lysing cells to release their contents for further research; outside the laboratory, sonication is used for cleaning surgical instruments, lenses, and a variety of other objects such as coins, tools, and musical instruments.

Filtration

Filtration is a method of physically separating microbes from samples. Air is commonly filtered through **high-efficiency particulate air (HEPA) filters** ([link](#)). HEPA filters have effective pore sizes of 0.3 μm , small enough to

capture bacterial cells, endospores, and many viruses, as air passes through these filters, nearly sterilizing the air on the other side of the filter. HEPA filters have a variety of applications and are used widely in clinical settings, in cars and airplanes, and even in the home. For example, they may be found in vacuum cleaners, heating and air-conditioning systems, and air purifiers.



(a) HEPA filters like this one remove microbes, endospores, and viruses as air flows through them. (b) A schematic of a HEPA filter. (credit a: modification of work by CSIRO; credit b: modification of work by “LadyofHats”/Mariana Ruiz Villareal)

Biological Safety Cabinets

Biological safety cabinets are a good example of the use of HEPA filters. HEPA filters in biological safety cabinets (BSCs) are used to remove particulates in the air either entering the cabinet (air intake), leaving the cabinet (air exhaust), or treating both the intake and exhaust. Use of an air-intake HEPA filter prevents environmental contaminants from entering the BSC, creating a clean area for handling biological materials. Use of an air-

exhaust HEPA filter prevents laboratory pathogens from contaminating the laboratory, thus maintaining a safe work area for laboratory personnel.

There are three classes of BSCs: I, II, and III. Each class is designed to provide a different level of protection for laboratory personnel and the environment; BSC II and III are also designed to protect the materials or devices in the cabinet. [\[link\]](#) summarizes the level of safety provided by each class of BSC for each BSL.

Biological Risks and BSCs				
Biological Risk Assessed	BSC Class	Protection of Personnel	Protection of Environment	Protection of Product
BSL-1, BSL-2, BSL-3	I	Yes	Yes	No
BSL-1, BSL-2, BSL-3	II	Yes	Yes	Yes
BSL-4	III; II when used in suit room with suit	Yes	Yes	Yes

Class I BSCs protect laboratory workers and the environment from a low to moderate risk for exposure to biological agents used in the laboratory. Air is drawn into the cabinet and then filtered before exiting through the building's exhaust system. Class II BSCs use directional air flow and partial barrier systems to contain infectious agents. Class III BSCs are designed for working with highly infectious agents like those used in BSL-4 laboratories. They are gas tight, and materials entering or exiting the cabinet must be passed through a double-door system, allowing the intervening space to be decontaminated between uses. All air is passed through one or two HEPA filters and an air incineration system before being exhausted directly to the outdoors (not through the building's exhaust system). Personnel can manipulate materials inside the Class III cabinet by using long rubber gloves sealed to the cabinet.

Note:

This [video](#) shows how BSCs are designed and explains how they protect personnel, the environment, and the product.

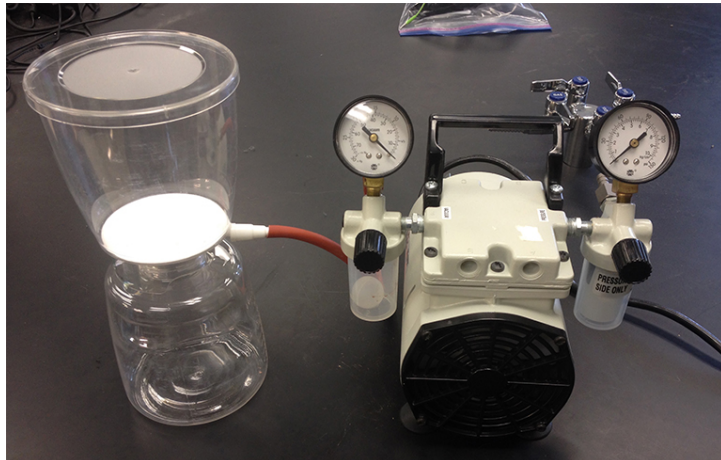
Filtration in Hospitals

HEPA filters are also commonly used in hospitals and surgical suites to prevent contamination and the spread of airborne microbes through ventilation systems. HEPA filtration systems may be designed for entire buildings or for individual rooms. For example, burn units, operating rooms, or isolation units may require special HEPA-filtration systems to remove opportunistic pathogens from the environment because patients in these rooms are particularly vulnerable to infection.

Membrane Filters

Filtration can also be used to remove microbes from liquid samples using **membrane filtration**. Membrane filters for liquids function similarly to HEPA filters for air. Typically, membrane filters that are used to remove bacteria have an effective pore size of 0.2 μm , smaller than the average size of a bacterium (1 μm), but filters with smaller pore sizes are available for more specific

needs. Membrane filtration is useful for removing bacteria from various types of heat-sensitive solutions used in the laboratory, such as antibiotic solutions and vitamin solutions. Large volumes of culture media may also be filter sterilized rather than autoclaved to protect heat-sensitive components. Often when filtering small volumes, syringe filters are used, but vacuum filters are typically used for filtering larger volumes ([link](#)).



(a)



(b)

Membrane filters come in a variety of sizes, depending on the volume of solution being filtered. (a) Larger volumes are filtered in units like these.

The solution is drawn through the filter by connecting the unit to a vacuum. (b) Smaller volumes are often filtered using syringe filters, which are units that fit on the end of a syringe. In this case, the solution is pushed through by depressing the syringe's plunger. (credit a, b: modification of work by Brian Forster)

Note:

- Would membrane filtration with a 0.2- μm filter likely remove viruses from a solution? Explain.
- Name at least two common uses of HEPA filtration in clinical or laboratory settings.

[\[link\]](#) and [\[link\]](#) summarize the physical methods of control discussed in this section.

Physical Methods of Control			
Method	Conditions	Mode of Action	Example Uses
Heat			
Boiling	100 °C at sea level	Denatures proteins and alters membranes	Cooking, personal use, preparing certain laboratory media
Dry-heat oven	170 °C for 2 hours	Denatures proteins and alters membranes, dehydration, desiccation	Sterilization of heat-stable medical and laboratory equipment and glassware
Incineration	Exposure to flame	Destroy by burning	Flaming loop, microincinerator
Autoclave	Typical settings: 121 °C for 15 minutes at 15 pounds per square inch (psi)	Denatures proteins and alters membranes	Sterilization of microbiological media, heat-stable medical and laboratory equipment, and other heat-stable items
Pasteurization	Can vary. One type is 72 °C for 15 seconds (HTST)	Denatures proteins and alters membranes	Prevents spoilage of milk, apple juice, honey, and other ingestible liquids
Cold			
Refrigeration	0 °C to 7 °C	Inhibits metabolism (slows or arrests cell division)	Preservation of food or laboratory materials (solutions, cultures)
Freezing	Below –2 °C	Stops metabolism, may kill microbes	Long-term storage of food, laboratory cultures, or medical specimens
Pressure			
High-pressure processing	100–800 MPa	Denatures proteins and can cause cell lysis	Preservation of food
Hyperbaric oxygen therapy	Air pressure three times higher than normal	Inhibits metabolism and growth of anaerobic microbes	Treatment of certain infections (e.g., gas gangrene)
Desiccation			
Simple desiccation	Drying	Inhibits metabolism	Dried fruits, jerky
Reduce water activity	Addition of salt or water	Inhibits metabolism and can cause lysis	Salted meats and fish, honey, jams and jellies
Lyophilization	Rapid freezing under vacuum	Inhibits metabolism	Preservation of food, laboratory cultures, or reagents
Radiation			
Ionizing radiation	Exposure to X-rays or gamma rays	Alters molecular structures, introduces double-strand breaks into DNA	Sterilization of spices and heat-sensitive laboratory and medical items; used for food sterilization in Europe but not widely accepted in US
Nonionizing radiation	Exposure to ultraviolet light	Introduces thymine dimers, leading to mutations	Disinfection of surfaces in laboratories and rooms in health-care environment, and disinfection of water and air

Physical Methods of Control (continued)			
Method	Conditions	Mode of Action	Example Uses
Sonication			
Sonication	Exposure to ultrasonic waves	Cavitation (formation of empty space) disrupts cells, lysing them	Laboratory research to lyse cells; cleaning jewelry, lenses, and equipment
Filtration			
HEPA filtration	Use of high-efficiency particulate air (HEPA) filter with 0.3 μm pore size	Physically removes microbes from air	Laboratory biological safety cabinets, operating rooms, isolation units, heating and air conditioning systems, vacuum cleaners
Membrane filtration	Use of membrane filter with 0.2- μm or smaller pore size	Physically removes microbes from liquid solutions	Removal of bacteria from heat-sensitive solutions like vitamins, antibiotics, and media with heat-sensitive components

Key Concepts and Summary

- Heat is a widely used and highly effective method for controlling microbial growth.
- **Dry-heat sterilization** protocols are used commonly in aseptic techniques in the laboratory. However, **moist-heat sterilization** is typically the more effective protocol because it penetrates cells better than dry heat does.
- **Pasteurization** is used to kill pathogens and reduce the number of microbes that cause food spoilage. **High-temperature, short-time pasteurization** is commonly used to pasteurize milk that will be refrigerated; **ultra-high temperature pasteurization** can be used to pasteurize milk for long-term storage without refrigeration.
- Refrigeration slows microbial growth; freezing stops growth, killing some organisms. Laboratory and medical specimens may be frozen on dry ice or at ultra-low temperatures for storage and transport.
- High-pressure processing can be used to kill microbes in food. Hyperbaric oxygen therapy to increase oxygen saturation has also been used to treat certain infections.
- **Desiccation** has long been used to preserve foods and is accelerated through the addition of salt or sugar, which decrease water activity in foods.
- **Lyophilization** combines cold exposure and desiccation for the long-term storage of foods and laboratory materials, but microbes remain and can be

rehydrated.

- **Ionizing radiation**, including gamma irradiation, is an effective way to sterilize heat-sensitive and packaged materials. **Nonionizing radiation**, like ultraviolet light, is unable to penetrate surfaces but is useful for surface sterilization.
- **HEPA** filtration is commonly used in hospital ventilation systems and biological safety cabinets in laboratories to prevent transmission of airborne microbes. **Membrane filtration** is commonly used to remove bacteria from heat-sensitive solutions.

Multiple Choice

Exercise:

Problem:

Which of the following methods brings about cell lysis due to cavitation induced by rapid localized pressure changes?

- A. microwaving
- B. gamma irradiation
- C. ultraviolet radiation
- D. sonication

Solution:

D

Exercise:

Problem:

Which of the following terms is used to describe the time required to kill all of the microbes within a sample at a given temperature?

- A. D-value
- B. thermal death point
- C. thermal death time
- D. decimal reduction time

Solution:

C

Exercise:**Problem:**

Which of the following microbial control methods does not actually kill microbes or inhibit their growth but instead removes them physically from samples?

- A. filtration
- B. desiccation
- C. lyophilization
- D. nonionizing radiation

Solution:

A

Fill in the Blank**Exercise:****Problem:**

In an autoclave, the application of pressure to _____ is increased to allow the steam to achieve temperatures above the boiling point of water.

Solution:

steam

True/False**Exercise:**

Problem:

Ionizing radiation can penetrate surfaces, but nonionizing radiation cannot.

Solution:

True

Exercise:

Problem:

Moist-heat sterilization protocols require the use of higher temperatures for longer periods of time than do dry-heat sterilization protocols do.

Solution:

False

Short Answer

Exercise:

Problem:

What is the advantage of HTST pasteurization compared with sterilization? What is an advantage of UHT treatment?

Exercise:

Problem: How does the addition of salt or sugar help preserve food?

Exercise:

Problem:

Which is more effective at killing microbes: autoclaving or freezing? Explain.

Critical Thinking

Exercise:

Problem:

In 2001, endospores of *Bacillus anthracis*, the causative agent of anthrax, were sent to government officials and news agencies via the mail. In response, the US Postal Service began to irradiate mail with UV light. Was this an effective strategy? Why or why not?

Using Chemicals to Control Microorganisms

LEARNING OBJECTIVES

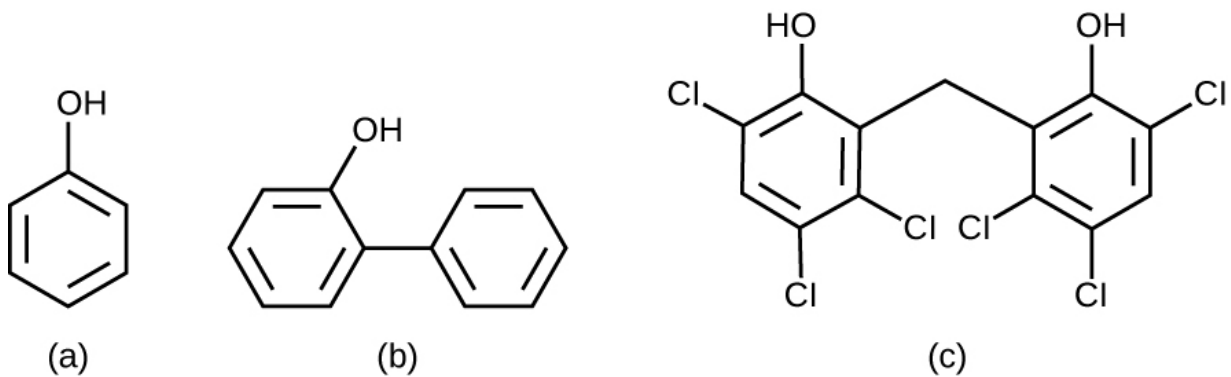
- Understand and compare various chemicals used to control microbial growth, including their uses, advantages and disadvantages, chemical structure, and mode of action

In addition to physical methods of microbial control, chemicals are also used to control microbial growth. A wide variety of chemicals can be used as disinfectants or antiseptics. When choosing which to use, it is important to consider the type of microbe targeted; how clean the item needs to be; the disinfectant's effect on the item's integrity; its safety to animals, humans, and the environment; its expense; and its ease of use. This section describes the variety of chemicals used as disinfectants and antiseptics, including their mechanisms of action and common uses.

Phenolics

In the 1800s, scientists began experimenting with a variety of chemicals for disinfection. In the 1860s, British surgeon Joseph Lister (1827–1912) began using carbolic acid, known as phenol, as a disinfectant for the treatment of surgical wounds (see [Foundations of Modern Cell Theory](#)). In 1879, Lister's work inspired the American chemist Joseph Lawrence (1836–1909) to develop Listerine, an alcohol-based mixture of several related compounds that is still used today as an oral antiseptic. Today, carbolic acid is no longer used as a surgical disinfectant because it is a skin irritant, but the chemical compounds found in antiseptic mouthwashes and throat lozenges are called **phenolics**.

Chemically, phenol consists of a benzene ring with an –OH group, and phenolics are compounds that have this group as part of their chemical structure ([\[link\]](#)). Phenolics such as thymol and eucalyptol occur naturally in plants. Other phenolics can be derived from creosote, a component of coal tar. Phenolics tend to be stable, persistent on surfaces, and less toxic than phenol. They inhibit microbial growth by denaturing proteins and disrupting membranes.



Phenol and phenolic compounds have been used to control microbial growth. (a) Chemical structure of phenol, also known as carbolic acid. (b) o-Phenylphenol, a type of phenolic, has been used as a disinfectant as well as to control bacterial and fungal growth on harvested citrus fruits. (c) Hexachlorophene, another phenol, known as a bisphenol (two rings), is the active ingredient in pHisoHex.

Since Lister's time, several phenolic compounds have been used to control microbial growth. Phenolics like cresols (methylated phenols) and o-phenylphenol were active ingredients in various formulations of Lysol since its invention in 1889. o-Phenylphenol was also commonly used in agriculture to control bacterial and fungal growth on harvested crops, especially citrus fruits, but its use in the United States is now far more limited. The bisphenol hexachlorophene, a disinfectant, is the active ingredient in pHisoHex, a topical cleansing detergent widely used for handwashing in hospital settings. pHisoHex is particularly effective against

gram-positive bacteria, including those causing staphylococcal and streptococcal skin infections. pHisoHex was formerly used for bathing infants, but this practice has been discontinued because it has been shown that exposure to hexachlorophene can lead to neurological problems.

Triclosan is another bisphenol compound that has seen widespread application in antibacterial products over the last several decades. Initially used in toothpastes, triclosan is now commonly used in hand soaps and is frequently impregnated into a wide variety of other products, including cutting boards, knives, shower curtains, clothing, and concrete, to make them antimicrobial. It is particularly effective against gram-positive bacteria on the skin, as well as certain gram-negative bacteria and yeasts.[\[footnote\]](#) US Food and Drug Administration. “Triclosan: What Consumers Should Know.” 2015.

<http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm205999.htm>. Accessed June 9, 2016.

Note:**Triclosan: Antibacterial Overkill?**

Hand soaps and other cleaning products are often marketed as “antibacterial,” suggesting that they provide a level of cleanliness superior to that of conventional soaps and cleansers. But are the antibacterial ingredients in these products really safe and effective?

About 75% of antibacterial liquid hand soaps and 30% of bar soaps contain the chemical triclosan, a phenolic, ([\[link\]](#)).[\[footnote\]](#) Triclosan blocks an enzyme in the bacterial fatty acid-biosynthesis pathway that is not found in the comparable human pathway. Although the use of triclosan in the home increased dramatically during the 1990s, more than 40 years of research by the FDA have turned up no conclusive evidence that washing with triclosan-containing products provides increased health benefits compared with washing with traditional soap. Although some studies indicate that fewer bacteria may remain on a person’s hands after washing with triclosan-based soap, compared with traditional soap, no evidence points to any reduction in the transmission of bacteria that cause respiratory and

gastrointestinal illness. In short, soaps with triclosan may remove or kill a few more germs but not enough to reduce the spread of disease.

J. Stromberg. “Five Reasons Why You Should Probably Stop Using Antibacterial Soap.” *Smithsonian.com* January 3, 2014.

<http://www.smithsonianmag.com/science-nature/five-reasons-why-you-should-probably-stop-using-antibacterial-soap-180948078/?no-ist>.

Accessed June 9, 2016.

Perhaps more disturbing, some clear risks associated with triclosan-based soaps have come to light. The widespread use of triclosan has led to an increase in triclosan-resistant bacterial strains, including those of clinical importance, such as *Salmonella enterica*; this resistance may render triclosan useless as an antibacterial in the long run.[\[footnote\]](#)[\[footnote\]](#)

Bacteria can easily gain resistance to triclosan through a change to a single gene encoding the targeted enzyme in the bacterial fatty acid-synthesis pathway. Other disinfectants with a less specific mode of action are much less prone to engendering resistance because it would take much more than a single genetic change.

SP Yazdankhah et al. “Triclosan and Antimicrobial Resistance in Bacteria: An Overview.” *Microbial Drug Resistance* 12 no. 2 (2006):83–90.

L. Birošová, M. Mikulášová. “Development of Triclosan and Antibiotic Resistance in *Salmonella enterica* serovar Typhimurium.” *Journal of Medical Microbiology* 58 no. 4 (2009):436–441.

Use of triclosan over the last several decades has also led to a buildup of the chemical in the environment. Triclosan in hand soap is directly introduced into wastewater and sewage systems as a result of the handwashing process. There, its antibacterial properties can inhibit or kill bacteria responsible for the decomposition of sewage, causing septic systems to clog and back up. Eventually, triclosan in wastewater finds its way into surface waters, streams, lakes, sediments, and soils, disrupting natural populations of bacteria that carry out important environmental functions, such as inhibiting algae. Triclosan also finds its way into the bodies of amphibians and fish, where it can act as an endocrine disruptor. Detectable levels of triclosan have also been found in various human bodily fluids, including breast milk, plasma, and urine.[\[footnote\]](#) In fact, a study conducted by the CDC found detectable levels of triclosan in the urine of 75% of 2,517 people tested in 2003–2004.[\[footnote\]](#) This finding

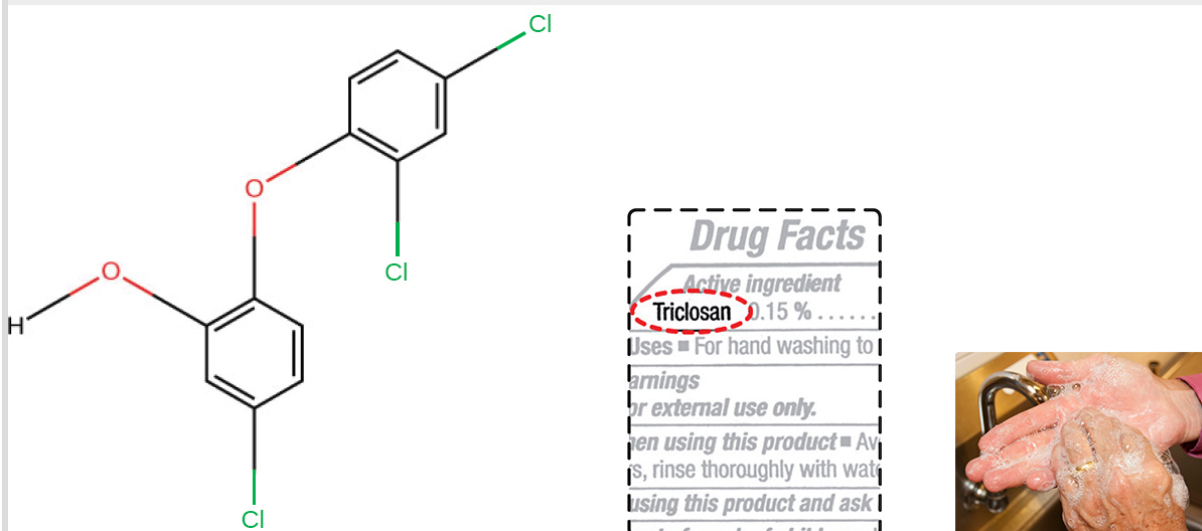
is even more troubling given the evidence that triclosan may affect immune function in humans. [\[footnote\]](#)

AB Dann, A. Hontela. "Triclosan: Environmental Exposure, Toxicity and Mechanisms of Action." *Journal of Applied Toxicology* 31 no. 4 (2011):285–311.

US Centers for Disease Control and Prevention. "Triclosan Fact Sheet." 2013. http://www.cdc.gov/biomonitoring/Triclosan_FactSheet.html. Accessed June 9, 2016.

EM Clayton et al. "The Impact of Bisphenol A and Triclosan on Immune Parameters in the US Population, NHANES 2003-2006." *Environmental Health Perspectives* 119 no. 3 (2011):390.

In December 2013, the FDA gave soap manufacturers until 2016 to prove that antibacterial soaps provide a significant benefit over traditional soaps; if unable to do so, manufacturers will be forced to remove these products from the market.



Triclosan is a common ingredient in antibacterial soaps despite evidence that it poses environmental and health risks and offers no significant health benefit compared to conventional soaps. (credit b, c: modification of work by FDA)

Note:

- Why is triclosan more like an antibiotic than a traditional disinfectant?

Heavy Metals

Some of the first chemical disinfectants and antiseptics to be used were heavy metals. Heavy metals kill microbes by binding to proteins, thus inhibiting enzymatic activity ([\[link\]](#)). Heavy metals are oligodynamic, meaning that very small concentrations show significant antimicrobial activity. Ions of heavy metals bind to sulfur-containing amino acids strongly and bioaccumulate within cells, allowing these metals to reach high localized concentrations. This causes proteins to denature.

Heavy metals are not selectively toxic to microbial cells. They may bioaccumulate in human or animal cells, as well, and excessive concentrations can have toxic effects on humans. If too much silver accumulates in the body, for example, it can result in a condition called argyria, in which the skin turns irreversibly blue-gray. One way to reduce the potential toxicity of heavy metals is by carefully controlling the duration of exposure and concentration of the heavy metal.



(a)



(b)



(c)



(d)



(e)

Heavy metals denature proteins, impairing cell function and, thus, giving them strong antimicrobial properties. (a) Copper in fixtures like

this door handle kills microbes that otherwise might accumulate on frequently touched surfaces. (b) Eating utensils contain small amounts of silver to inhibit microbial growth. (c) Copper commonly lines incubators to minimize contamination of cell cultures stored inside. (d) Antiseptic mouthwashes commonly contain zinc chloride. (e) This patient is suffering from argyria, an irreversible condition caused by bioaccumulation of silver in the body. (credit b: modification of work by “Shoshanah”/Flickr; credit e: modification of work by Herbert L. Fred and Hendrik A. van Dijk)

Mercury

Mercury is an example of a heavy metal that has been used for many years to control microbial growth. It was used for many centuries to treat syphilis. Mercury compounds like mercuric chloride are mainly bacteriostatic and have a very broad spectrum of activity. Various forms of mercury bind to sulfur-containing amino acids within proteins, inhibiting their functions.

In recent decades, the use of such compounds has diminished because of mercury's toxicity. It is toxic to the central nervous, digestive, and renal systems at high concentrations, and has negative environmental effects, including bioaccumulation in fish. Topical antiseptics such as mercurochrome, which contains mercury in low concentrations, and merthiolate, a **tincture** (a solution of mercury dissolved in alcohol) were once commonly used. However, because of concerns about using mercury compounds, these antiseptics are no longer sold in the United States.

Silver

Silver has long been used as an antiseptic. In ancient times, drinking water was stored in silver jugs. [\[footnote\]](#) Silvadene cream is commonly used to treat topical wounds and is particularly helpful in preventing infection in

burn wounds. Silver nitrate drops were once routinely applied to the eyes of newborns to protect against ophthalmia neonatorum, eye infections that can occur due to exposure to pathogens in the birth canal, but antibiotic creams are more now commonly used. Silver is often combined with antibiotics, making the antibiotics thousands of times more effective.[\[footnote\]](#) Silver is also commonly incorporated into catheters and bandages, rendering them antimicrobial; however, there is evidence that heavy metals may also enhance selection for antibiotic resistance.[\[footnote\]](#)

N. Silvestry-Rodriguez et al. "Silver as a Disinfectant." In *Reviews of Environmental Contamination and Toxicology*, pp. 23-45. Edited by GW Ware and DM Whitacre. New York: Springer, 2007.

B. Owens. "Silver Makes Antibiotics Thousands of Times More Effective." *Nature* June 19 2013. <http://www.nature.com/news/silver-makes-antibiotics-thousands-of-times-more-effective-1.13232>

C. Seiler, TU Berendonk. "Heavy Metal Driven Co-Selection of Antibiotic Resistance in Soil and Water Bodies Impacted by Agriculture and Aquaculture." *Frontiers in Microbiology* 3 (2012):399.

Copper, Nickel, and Zinc

Several other heavy metals also exhibit antimicrobial activity. Copper sulfate is a common algicide used to control algal growth in swimming pools and fish tanks. The use of metallic copper to minimize microbial growth is also becoming more widespread. Copper linings in incubators help reduce contamination of cell cultures. The use of copper pots for water storage in underdeveloped countries is being investigated as a way to combat diarrheal diseases. Copper coatings are also becoming popular for frequently handled objects such as doorknobs, cabinet hardware, and other fixtures in health-care facilities in an attempt to reduce the spread of microbes.

Nickel and zinc coatings are now being used in a similar way. Other forms of zinc, including zinc chloride and zinc oxide, are also used commercially. Zinc chloride is quite safe for humans and is commonly found in mouthwashes, substantially increasing their length of effectiveness. Zinc oxide is found in a variety of products, including topical antiseptic creams

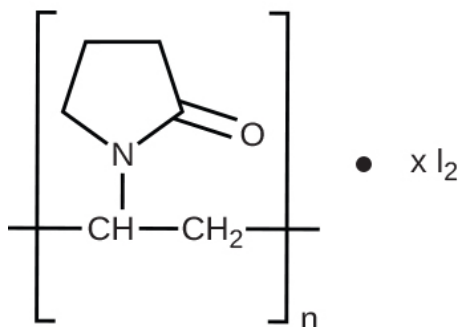
such as calamine lotion, diaper ointments, baby powder, and dandruff shampoos.

Note:

- Why are many heavy metals both antimicrobial and toxic to humans?

Halogens

Other chemicals commonly used for disinfection are the halogens iodine, chlorine, and fluorine. Iodine works by oxidizing cellular components, including sulfur-containing amino acids, nucleotides, and fatty acids, and destabilizing the macromolecules that contain these molecules. It is often used as a topical tincture, but it may cause staining or skin irritation. An **iodophor** is a compound of iodine complexed with an organic molecule, thereby increasing iodine's stability and, in turn, its efficacy. One common iodophor is povidone-iodine, which includes a wetting agent that releases iodine relatively slowly. Betadine is a brand of povidone-iodine commonly used as a hand scrub by medical personnel before surgery and for topical antisepsis of a patient's skin before incision ([\[link\]](#)).



(a)



(b)

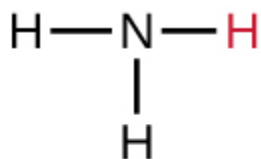
(a) Betadine is a solution of the iodophor povidone-iodine. (b) It is commonly used as a topical antiseptic on a patient's skin before incision during surgery. (credit b: modification of work by Andrew Ratto)

Chlorine is another halogen commonly used for disinfection. When chlorine gas is mixed with water, it produces a strong oxidant called hypochlorous acid, which is uncharged and enters cells easily. Chlorine gas is commonly used in municipal drinking water and wastewater treatment plants, with the resulting hypochlorous acid producing the actual antimicrobial effect. Those working at water treatment facilities need to take great care to minimize personal exposure to chlorine gas. Sodium hypochlorite is the chemical component of common household bleach, and it is also used for a wide variety of disinfecting purposes. Hypochlorite salts, including sodium and calcium hypochlorites, are used to disinfect swimming pools. Chlorine gas, sodium hypochlorite, and calcium hypochlorite are also commonly used disinfectants in the food processing and restaurant industries to reduce the spread of foodborne diseases. Workers in these industries also need to take care to use these products correctly to ensure their own safety as well as the safety of consumers. A recent joint statement published by the Food and Agriculture Organization (FAO) of the United Nations and WHO indicated that none of the many beneficial uses of chlorine products in food

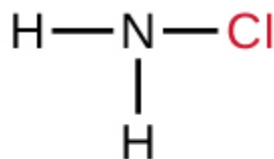
processing to reduce the spread of foodborne illness posed risks to consumers.[\[footnote\]](#)

World Health Organization. “Benefits and Risks of the Use of Chlorine-Containing Disinfectants in Food Production and Food Processing: Report of a Joint FAO/WHO Expert Meeting.” Geneva, Switzerland: World Health Organization, 2009.

Another class of chlorinated compounds called chloramines are widely used as disinfectants. Chloramines are relatively stable, releasing chlorine over long periods time. Chloramines are derivatives of ammonia by substitution of one, two, or all three hydrogen atoms with chlorine atoms ([\[link\]](#)).



ammonia



chloramine

Monochloroamine, one of the chloramines, is derived from ammonia by the replacement of one hydrogen atom with a chlorine atom.

Chloramines and other chlorine compounds may be used for disinfection of drinking water, and chloramine tablets are frequently used by the military for this purpose. After a natural disaster or other event that compromises the public water supply, the CDC recommends disinfecting tap water by adding small amounts of regular household bleach. Recent research suggests that sodium dichloroisocyanurate (NaDCC) may also be a good alternative for drinking water disinfection. Currently, NaDCC tablets are available for general use and for use by the military, campers, or those with emergency needs; for these uses, NaDCC is preferable to chloramine tablets. Chlorine

dioxide, a gaseous agent used for fumigation and sterilization of enclosed areas, is also commonly used for the disinfection of water.

Although chlorinated compounds are relatively effective disinfectants, they have their disadvantages. Some may irritate the skin, nose, or eyes of some individuals, and they may not completely eliminate certain hardy organisms from contaminated drinking water. The protozoan parasite *Cryptosporidium*, for example, has a protective outer shell that makes it resistant to chlorinated disinfectants. Thus, boiling of drinking water in emergency situations is recommended when possible.

The halogen fluorine is also known to have antimicrobial properties that contribute to the prevention of dental caries (cavities).[\[footnote\]](#) Fluoride is the main active ingredient of toothpaste and is also commonly added to tap water to help communities maintain oral health. Chemically, fluoride can become incorporated into the hydroxyapatite of tooth enamel, making it more resistant to corrosive acids produced by the fermentation of oral microbes. Fluoride also enhances the uptake of calcium and phosphate ions in tooth enamel, promoting remineralization. In addition to strengthening enamel, fluoride also seems to be bacteriostatic. It accumulates in plaque-forming bacteria, interfering with their metabolism and reducing their production of the acids that contribute to tooth decay.

RE Marquis. “Antimicrobial Actions of Fluoride for Oral Bacteria.” *Canadian Journal of Microbiology* 41 no. 11 (1995):955–964.

Note:

- What is a benefit of a chloramine over hypochlorite for disinfecting?

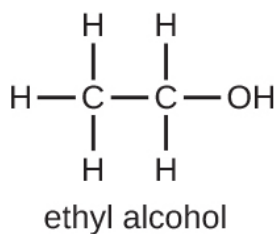
Alcohols

Alcohols make up another group of chemicals commonly used as disinfectants and antiseptics. They work by rapidly denaturing proteins,

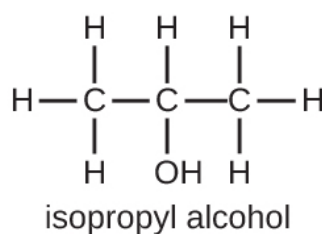
which inhibits cell metabolism, and by disrupting membranes, which leads to cell lysis. Once denatured, the proteins may potentially refold if enough water is present in the solution. Alcohols are typically used at concentrations of about 70% aqueous solution and, in fact, work better in aqueous solutions than 100% alcohol solutions. This is because alcohols coagulate proteins. In higher alcohol concentrations, rapid coagulation of surface proteins prevents effective penetration of cells. The most commonly used alcohols for disinfection are ethyl alcohol (ethanol) and isopropyl alcohol (isopropanol, rubbing alcohol) ([link](#)).

Alcohols tend to be bactericidal and fungicidal, but may also be viricidal for enveloped viruses only. Although alcohols are not sporicidal, they do inhibit the processes of sporulation and germination. Alcohols are volatile and dry quickly, but they may also cause skin irritation because they dehydrate the skin at the site of application. One common clinical use of alcohols is swabbing the skin for degerming before needle injection. Alcohols also are the active ingredients in instant hand sanitizers, which have gained popularity in recent years. The alcohol in these hand sanitizers works both by denaturing proteins and by disrupting the microbial cell membrane, but will not work effectively in the presence of visible dirt.

Last, alcohols are used to make tinctures with other antiseptics, such as the iodine tinctures discussed previously in this chapter. All in all, alcohols are inexpensive and quite effective for the disinfection of a broad range of vegetative microbes. However, one disadvantage of alcohols is their high volatility, limiting their effectiveness to immediately after application.



(a)



(b)

(a) Ethyl alcohol, the intoxicating ingredient found in alcoholic drinks, is also used commonly as a disinfectant. (b) Isopropyl alcohol, also called rubbing alcohol, has a related molecular structure and is another commonly used disinfectant. (credit a photo: modification of work by D Coetzee; credit b photo: modification of work by Craig Spurrier)

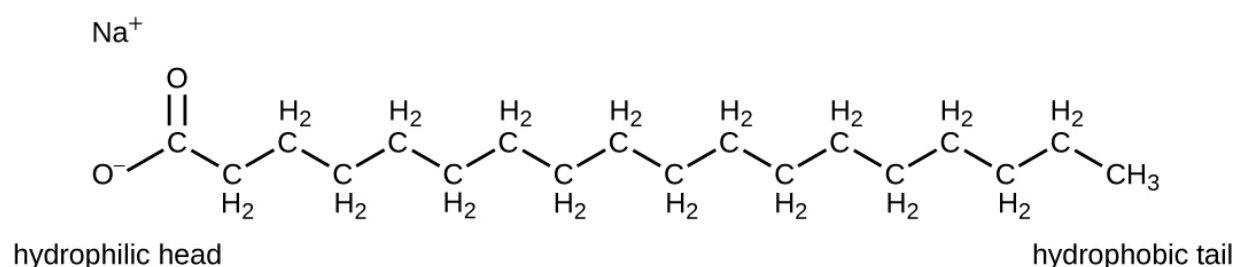
Note:

- Name at least three advantages of alcohols as disinfectants.
- Describe several specific applications of alcohols used in disinfectant products.

Surfactants

Surface-active agents, or **surfactants**, are a group of chemical compounds that lower the surface tension of water. Surfactants are the major ingredients in soaps and detergents. Soaps are salts of long-chain fatty acids and have both polar and nonpolar regions, allowing them to interact with polar and

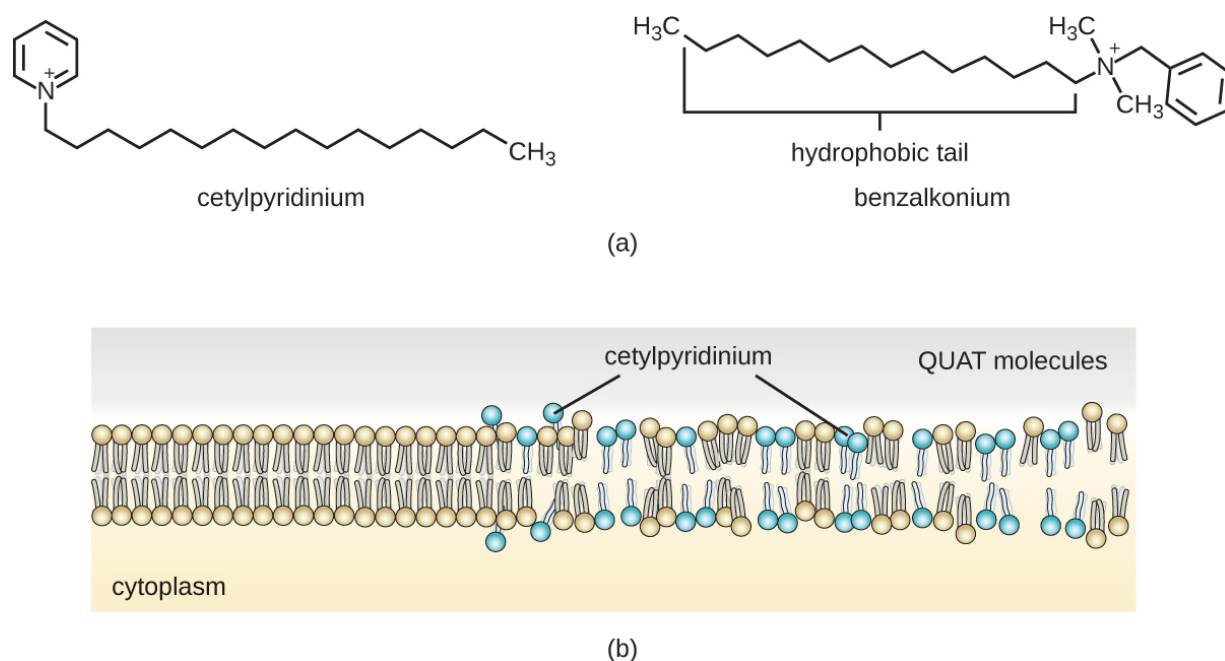
nonpolar regions in other molecules ([\[link\]](#)). They can interact with nonpolar oils and grease to create emulsions in water, loosening and lifting away dirt and microbes from surfaces and skin. Soaps do not kill or inhibit microbial growth and so are not considered antiseptics or disinfectants. However, proper use of soaps mechanically carries away microorganisms, effectively degerming a surface. Some soaps contain added bacteriostatic agents such as triclocarban or cloflucarban, compounds structurally related to triclosan, that introduce antiseptic or disinfectant properties to the soaps.



Soaps are the salts (sodium salt in the illustration) of fatty acids and have the ability to emulsify lipids, fats, and oils by interacting with water through their hydrophilic heads and with the lipid at their hydrophobic tails.

Soaps, however, often form films that are difficult to rinse away, especially in hard water, which contains high concentrations of calcium and magnesium mineral salts. Detergents contain synthetic surfactant molecules with both polar and nonpolar regions that have strong cleansing activity but are more soluble, even in hard water, and, therefore, leave behind no soapy deposits. Anionic detergents, such as those used for laundry, have a negatively charged anion at one end attached to a long hydrophobic chain, whereas cationic detergents have a positively charged cation instead. Cationic detergents include an important class of disinfectants and antiseptics called the **quaternary ammonium salts (quats)**, named for the characteristic quaternary nitrogen atom that confers the positive charge ([\[link\]](#)). Overall, quats have properties similar to phospholipids, having hydrophilic and hydrophobic ends. As such, quats have the ability to insert

into the bacterial phospholipid bilayer and disrupt membrane integrity. The cationic charge of quats appears to confer their antimicrobial properties, which are diminished when neutralized. Quats have several useful properties. They are stable, nontoxic, inexpensive, colorless, odorless, and tasteless. They tend to be bactericidal by disrupting membranes. They are also active against fungi, protozoans, and enveloped viruses, but endospores are unaffected. In clinical settings, they may be used as antiseptics or to disinfect surfaces. Mixtures of quats are also commonly found in household cleaners and disinfectants, including many current formulations of Lysol brand products, which contain benzalkonium chlorides as the active ingredients. Benzalkonium chlorides, along with the quat cetylpyrimidine chloride, are also found in products such as skin antiseptics, oral rinses, and mouthwashes.



(a) Two common quats are benzylalkonium chloride and cetylpyrimidine chloride. Note the hydrophobic nonpolar carbon chain at one end and the nitrogen-containing cationic component at the other end. (b) Quats are able to infiltrate the phospholipid plasma membranes of bacterial cells and disrupt their integrity, leading to death of the cell.

Note:

- Why are soaps not considered disinfectants?

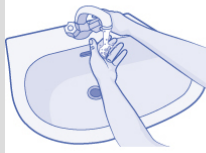
Note:**Handwashing the Right Way**

Handwashing is critical for public health and should be emphasized in a clinical setting. For the general public, the CDC recommends handwashing before, during, and after food handling; before eating; before and after interacting with someone who is ill; before and after treating a wound; after using the toilet or changing diapers; after coughing, sneezing, or blowing the nose; after handling garbage; and after interacting with an animal, its feed, or its waste. [\[link\]](#) illustrates the five steps of proper handwashing recommended by the CDC.

Handwashing is even more important for health-care workers, who should wash their hands thoroughly between every patient contact, after the removal of gloves, after contact with bodily fluids and potentially infectious fomites, and before and after assisting a surgeon with invasive procedures. Even with the use of proper surgical attire, including gloves, scrubbing for surgery is more involved than routine handwashing. The goal of surgical scrubbing is to reduce the normal microbiota on the skin's surface to prevent the introduction of these microbes into a patient's surgical wounds.

There is no single widely accepted protocol for surgical scrubbing. Protocols for length of time spent scrubbing may depend on the antimicrobial used; health-care workers should always check the manufacturer's recommendations. According to the Association of Surgical Technologists (AST), surgical scrubs may be performed with or without the use of brushes ([\[link\]](#)).

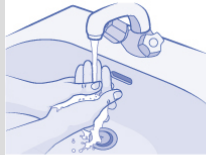
CDC handwashing recommendations for the general public



- 1 Wet** your hands with clean, running water (warm or cold), turn off the tap, and apply soap.



- 2 Lather** your hands by rubbing them together with the soap. Be sure to lather the backs of your hands, between your fingers, and under your nails.



- 3 Scrub** your hands for at least 20 seconds. Need a timer? Hum the “Happy Birthday” song from beginning to end twice.



- 4 Rinse** your hands well under clean, running water.



- 5 Dry** your hands using a clean towel or air-dry them.

(a)



(b)

(a) The CDC recommends five steps as part of typical handwashing for the general public. (b) Surgical scrubbing is more extensive, requiring scrubbing starting from the fingertips, extending to the hands and forearms, and then up beyond the elbows, as shown here. (credit a: modification of work by World Health Organization)

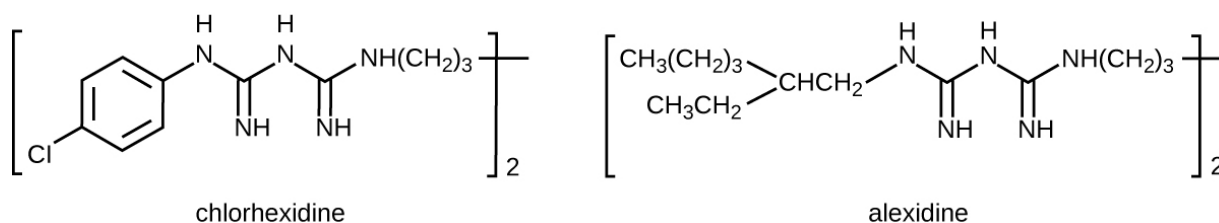
Note:

To [learn more](#) about proper handwashing, visit the CDC’s website.

Bisbiguanides

Bisbiguanides were first synthesized in the 20th century and are cationic (positively charged) molecules known for their antiseptic properties ([\[link\]](#)). One important **bisbiguanide** antiseptic is chlorhexidine. It has broad-spectrum activity against yeasts, gram-positive bacteria, and gram-negative bacteria, with the exception of *Pseudomonas aeruginosa*, which may develop resistance on repeated exposure. [\[footnote\]](#) Chlorhexidine disrupts cell membranes and is bacteriostatic at lower concentrations or bactericidal at higher concentrations, in which it actually causes the cells' cytoplasmic contents to congeal. It also has activity against enveloped viruses. However, chlorhexidine is poorly effective against *Mycobacterium tuberculosis* and nonenveloped viruses, and it is not sporicidal. Chlorhexidine is typically used in the clinical setting as a surgical scrub and for other handwashing needs for medical personnel, as well as for topical antiseptics for patients before surgery or needle injection. It is more persistent than iodophors, providing long-lasting antimicrobial activity. Chlorhexidine solutions may also be used as oral rinses after oral procedures or to treat gingivitis. Another bisbiguanide, alexidine, is gaining popularity as a surgical scrub and an oral rinse because it acts faster than chlorhexidine.

L. Thomas et al. "Development of Resistance to Chlorhexidine Diacetate in *Pseudomonas aeruginosa* and the Effect of a 'Residual' Concentration." *Journal of Hospital Infection* 46 no. 4 (2000):297–303.



The bisbiguanides chlorhexidine and alexidine are cationic antiseptic compounds commonly used as surgical scrubs.

Note:

- What two effects does chlorhexidine have on bacterial cells?

Alkylating Agents

The **alkylating agents** are a group of strong disinfecting chemicals that act by replacing a hydrogen atom within a molecule with an alkyl group (C_nH_{2n+1}), thereby inactivating enzymes and nucleic acids ([link](#)). The alkylating agent formaldehyde (CH_2OH) is commonly used in solution at a concentration of 37% (known as formalin) or as a gaseous disinfectant and biocide. It is a strong, broad-spectrum disinfectant and biocide that has the ability to kill bacteria, viruses, fungi, and endospores, leading to sterilization at low temperatures, which is sometimes a convenient alternative to the more labor-intensive heat sterilization methods. It also cross-links proteins and has been widely used as a chemical fixative. Because of this, it is used for the storage of tissue specimens and as an embalming fluid. It also has been used to inactivate infectious agents in vaccine preparation. Formaldehyde is very irritating to living tissues and is also carcinogenic; therefore, it is not used as an antiseptic.

Glutaraldehyde is structurally similar to formaldehyde but has two reactive aldehyde groups, allowing it to act more quickly than formaldehyde. It is commonly used as a 2% solution for sterilization and is marketed under the brand name Cidex. It is used to disinfect a variety of surfaces and surgical and medical equipment. However, similar to formaldehyde, glutaraldehyde irritates the skin and is not used as an antiseptic.

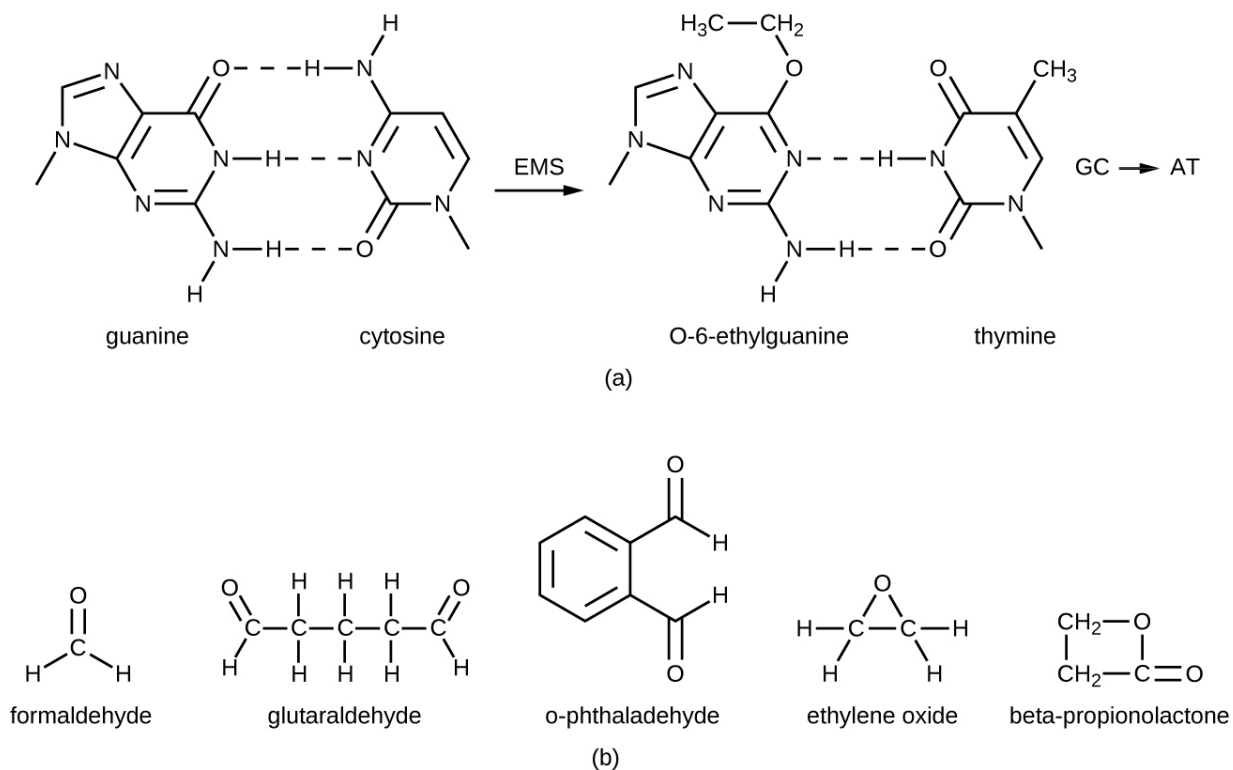
A new type of disinfectant gaining popularity for the disinfection of medical equipment is o-phthalaldehyde (OPA), which is found in some newer formulations of Cidex and similar products, replacing glutaraldehyde. o-Phthalaldehyde also has two reactive aldehyde groups, but they are linked by an aromatic bridge. o-Phthalaldehyde is thought to work similarly to glutaraldehyde and formaldehyde, but is much less

irritating to skin and nasal passages, produces a minimal odor, does not require processing before use, and is more effective against mycobacteria.

Ethylene oxide is a type of alkylating agent that is used for gaseous sterilization. It is highly penetrating and can sterilize items within plastic bags such as catheters, disposable items in laboratories and clinical settings (like packaged Petri dishes), and other pieces of equipment. Ethylene oxide exposure is a form of cold sterilization, making it useful for the sterilization of heat-sensitive items. Great care needs to be taken with the use of ethylene oxide, however; it is carcinogenic, like the other alkylating agents, and is also highly explosive. With careful use and proper aeration of the products after treatment, ethylene oxide is highly effective, and ethylene oxide sterilizers are commonly found in medical settings for sterilizing packaged materials.

β -Propionolactone is an alkylating agent with a different chemical structure than the others already discussed. Like other alkylating agents, β -propionolactone binds to DNA, thereby inactivating it ([\[link\]](#)). It is a clear liquid with a strong odor and has the ability to kill endospores. As such, it has been used in either liquid form or as a vapor for the sterilization of medical instruments and tissue grafts, and it is a common component of vaccines, used to maintain their sterility. It has also been used for the sterilization of nutrient broth, as well as blood plasma, milk, and water. It is quickly metabolized by animals and humans to lactic acid. It is also an irritant, however, and may lead to permanent damage of the eyes, kidneys, or liver. Additionally, it has been shown to be carcinogenic in animals; thus, precautions are necessary to minimize human exposure to β -propionolactone.[\[footnote\]](#)

Institute of Medicine. "Long-Term Health Effects of Participation in Project SHAD (Shipboard Hazard and Defense)." Washington, DC: The National Academies Press, 2007.



(a) Alkylating agents replace hydrogen atoms with alkyl groups. Here, guanine is alkylated, resulting in its hydrogen bonding with thymine, instead of cytosine. (b) The chemical structures of several alkylating agents.

Note:

- What chemical reaction do alkylating agents participate in?
- Why are alkylating agents not used as antiseptics?

Note:

Diehard Prions

Prions, the acellular, misfolded proteins responsible for incurable and fatal diseases such as kuru and Creutzfeldt-Jakob disease (see [Viroids](#), [Virusoids](#), and [Prions](#)), are notoriously difficult to destroy. Prions are extremely resistant to heat, chemicals, and radiation. They are also extremely infectious and deadly; thus, handling and disposing of prion-infected items requires extensive training and extreme caution. Typical methods of disinfection can reduce but not eliminate the infectivity of prions. Autoclaving is not completely effective, nor are chemicals such as phenol, alcohols, formalin, and β -propiolactone. Even when fixed in formalin, affected brain and spinal cord tissues remain infectious. Personnel who handle contaminated specimens or equipment or work with infected patients must wear a protective coat, face protection, and cut-resistant gloves. Any contact with skin must be immediately washed with detergent and warm water without scrubbing. The skin should then be washed with 1 N NaOH or a 1:10 dilution of bleach for 1 minute. Contaminated waste must be incinerated or autoclaved in a strong basic solution, and instruments must be cleaned and soaked in a strong basic solution.

Note:

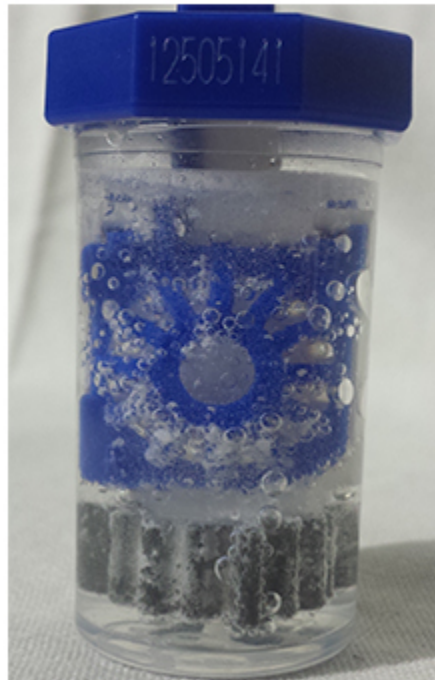
For more information on the handling of animals and prion-contaminated materials, visit the guidelines published on the [CDC](#) and [WHO](#) websites.

Peroxygens

Peroxygens are strong oxidizing agents that can be used as disinfectants or antiseptics. The most widely used **peroxygen** is hydrogen peroxide (H_2O_2), which is often used in solution to disinfect surfaces and may also be used as a gaseous agent. Hydrogen peroxide solutions are inexpensive skin antiseptics that break down into water and oxygen gas, both of which are environmentally safe. This decomposition is accelerated in the presence of light, so hydrogen peroxide solutions typically are sold in brown or opaque bottles. One disadvantage of using hydrogen peroxide as an antiseptic is

that it also causes damage to skin that may delay healing or lead to scarring. Contact lens cleaners often include hydrogen peroxide as a disinfectant.

Hydrogen peroxide works by producing free radicals that damage cellular macromolecules. Hydrogen peroxide has broad-spectrum activity, working against gram-positive and gram-negative bacteria (with slightly greater efficacy against gram-positive bacteria), fungi, viruses, and endospores. However, bacteria that produce the oxygen-detoxifying enzymes catalase or peroxidase may have inherent tolerance to low hydrogen peroxide concentrations ([link](#)). To kill endospores, the length of exposure or concentration of solutions of hydrogen peroxide must be increased. Gaseous hydrogen peroxide has greater efficacy and can be used as a sterilant for rooms or equipment.



Catalase enzymatically converts highly reactive hydrogen peroxide (H_2O_2) into water and oxygen.

Hydrogen peroxide can be used to clean wounds.
Hydrogen peroxide is used to sterilize items such
as contact lenses. (credit photos: modification of
work by Kerry Ceszyk)

Plasma, a hot, ionized gas, described as the fourth state of matter, is useful for sterilizing equipment because it penetrates surfaces and kills vegetative cells and endospores. Hydrogen peroxide and peracetic acid, another commonly used peroxygen, each may be introduced as a plasma. Peracetic acid can be used as a liquid or plasma sterilant insofar as it readily kills endospores, is more effective than hydrogen peroxide even at rather low concentrations, and is immune to inactivation by catalases and peroxidases. It also breaks down to environmentally innocuous compounds; in this case, acetic acid and oxygen.

Other examples of peroxygens include benzoyl peroxide and carbamide peroxide. Benzoyl peroxide is a peroxygen that used in acne medication solutions. It kills the bacterium *Propionibacterium acnes*, which is associated with acne. Carbamide peroxide, an ingredient used in toothpaste, is a peroxygen that combats oral biofilms that cause tooth discoloration and halitosis (bad breath).[\[footnote\]](#) Last, ozone gas is a peroxygen with disinfectant qualities and is used to clean air or water supplies. Overall, peroxygens are highly effective and commonly used, with no associated environmental hazard.

Yao, C.S. et al. “In vitro antibacterial effect of carbamide peroxide on oral biofilm.” *Journal of Oral Microbiology* Jun 12, 2013.
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3682087/>. doi:
10.3402/jom.v5i0.20392.

Note:

- How do peroxides kill cells?

Supercritical Fluids

Within the last 15 years, the use of **supercritical fluids**, especially supercritical carbon dioxide (scCO_2), has gained popularity for certain sterilizing applications. When carbon dioxide is brought to approximately 10 times atmospheric pressure, it reaches a supercritical state that has physical properties between those of liquids and gases. Materials put into a chamber in which carbon dioxide is pressurized in this way can be sterilized because of the ability of scCO_2 to penetrate surfaces.

Supercritical carbon dioxide works by penetrating cells and forming carbonic acid, thereby lowering the cell pH considerably. This technique is effective against vegetative cells and is also used in combination with peracetic acid to kill endospores. Its efficacy can also be augmented with increased temperature or by rapid cycles of pressurization and depressurization, which more likely produce cell lysis.

Benefits of scCO_2 include the nonreactive, nontoxic, and nonflammable properties of carbon dioxide, and this protocol is effective at low temperatures. Unlike other methods, such as heat and irradiation, that can degrade the object being sterilized, the use of scCO_2 preserves the object's integrity and is commonly used for treating foods (including spices and juices) and medical devices such as endoscopes. It is also gaining popularity for disinfecting tissues such as skin, bones, tendons, and ligaments prior to transplantation. scCO_2 can also be used for pest control because it can kill insect eggs and larvae within products.

Note:

- Why is the use of supercritical carbon dioxide gaining popularity for commercial and medical uses?

Chemical Food Preservatives

Chemical preservatives are used to inhibit microbial growth and minimize spoilage in some foods. Commonly used chemical preservatives include sorbic acid, benzoic acid, and propionic acid, and their more soluble salts potassium sorbate, sodium benzoate, and calcium propionate, all of which are used to control the growth of molds in acidic foods. Each of these preservatives is nontoxic and readily metabolized by humans. They are also flavorless, so they do not compromise the flavor of the foods they preserve.

Sorbic and benzoic acids exhibit increased efficacy as the pH decreases. Sorbic acid is thought to work by inhibiting various cellular enzymes, including those in the citric acid cycle, as well as catalases and peroxidases. It is added as a preservative in a wide variety of foods, including dairy, bread, fruit, and vegetable products. Benzoic acid is found naturally in many types of fruits and berries, spices, and fermented products. It is thought to work by decreasing intracellular pH, interfering with mechanisms such as oxidative phosphorylation and the uptake of molecules such as amino acids into cells. Foods preserved with benzoic acid or sodium benzoate include fruit juices, jams, ice creams, pastries, soft drinks, chewing gum, and pickles.

Propionic acid is thought to both inhibit enzymes and decrease intracellular pH, working similarly to benzoic acid. However, propionic acid is a more effective preservative at a higher pH than either sorbic acid or benzoic acid. Propionic acid is naturally produced by some cheeses during their ripening and is added to other types of cheese and baked goods to prevent mold contamination. It is also added to raw dough to prevent contamination by the bacterium *Bacillus mesentericus*, which causes bread to become roty.

Other commonly used chemical preservatives include sulfur dioxide and nitrites. Sulfur dioxide prevents browning of foods and is used for the preservation of dried fruits; it has been used in winemaking since ancient times. Sulfur dioxide gas dissolves in water readily, forming sulfites. Although sulfites can be metabolized by the body, some people have sulfite allergies, including asthmatic reactions. Additionally, sulfites degrade thiamine, an important nutrient in some foods. The mode of action of sulfites is not entirely clear, but they may interfere with the disulfide bond (see [\[link\]](#)) formation in proteins, inhibiting enzymatic activity.

Alternatively, they may reduce the intracellular pH of the cell, interfering with proton motive force-driven mechanisms.

Nitrites are added to processed meats to maintain color and stop the germination of *Clostridium botulinum* endospores. Nitrites are reduced to nitric oxide, which reacts with heme groups and iron-sulfur groups. When nitric oxide reacts with the heme group within the myoglobin of meats, a red product forms, giving meat its red color. Alternatively, it is thought that when nitric acid reacts with the iron-sulfur enzyme ferredoxin within bacteria, this electron transport-chain carrier is destroyed, preventing ATP synthesis. Nitrosamines, however, are carcinogenic and can be produced through exposure of nitrite-preserved meats (e.g., hot dogs, lunch meat, breakfast sausage, bacon, meat in canned soups) to heat during cooking.

Natural Chemical Food Preservatives

The discovery of natural antimicrobial substances produced by other microbes has added to the arsenal of preservatives used in food. Nisin is an antimicrobial peptide produced by the bacterium *Lactococcus lactis* and is particularly effective against gram-positive organisms. Nisin works by disrupting cell wall production, leaving cells more prone to lysis. It is used to preserve cheeses, meats, and beverages.

Natamycin is an antifungal macrolide antibiotic produced by the bacterium *Streptomyces natalensis*. It was approved by the FDA in 1982 and is used to prevent fungal growth in various types of dairy products, including cottage cheese, sliced cheese, and shredded cheese. Natamycin is also used for meat preservation in countries outside the United States.

Note:

- What are the advantages and drawbacks of using sulfites and nitrites as food preservatives?

Chemical Disinfectants		
Chemical	Mode of Action	Example Uses
Phenolics		
Cresols o-Phenylphenol Hexachlorophene Triclosan	Denature proteins and disrupt membranes	Disinfectant in Lysol Prevent contamination of crops (citrus) Antibacterial soap pHisoHex for handwashing in hospitals
Metals		

Chemical Disinfectants		
Chemical	Mode of Action	Example Uses
Mercury Silver Copper Nickel Zinc	Bind to proteins and inhibit enzyme activity	Topical antiseptic Treatment of wounds and burns Prevention of eye infections in newborns Antibacterial in catheters and bandages Mouthwash Algicide for pools and fish tanks Containers for long-term water storage
Halogens		

Chemical Disinfectants		
Chemical	Mode of Action	Example Uses
Iodine Chlorine Fluorine	Oxidation and destabilization of cellular macromolecules	Topical antiseptic Hand scrub for medical personnel Water disinfectant Water treatment plants Household bleach Food processing Prevention of dental carries
Alcohols		
Ethanol Isopropanol	Denature proteins and disrupt membranes	Disinfectant Antiseptic
Surfactants		
Quaternary ammonium salts	Lowers surface tension of water to help with washing away of microbes, and disruption of cell membranes	Soaps and detergent Disinfectant Antiseptic Mouthwash
Bisbiguanides		

Chemical Disinfectants		
Chemical	Mode of Action	Example Uses
Chlorhexidine Alexidine	Disruption of cell membranes	Oral rinse Hand scrub for medical personnel
Alkylating Agents		
Formaldehyde Glutaraldehyde o-Phthalaldehyde Ethylene oxide β -Propionolactone	Inactivation of enzymes and nucleic acid	Disinfectant Tissue specimen storage Embalming Sterilization of medical equipment Vaccine component for sterility
Peroxygens		
Hydrogen peroxide Peracetic acid Benzoyl peroxide Carbamide peroxide Ozone gas	Oxidation and destabilization of cellular macromolecules	Antiseptic Disinfectant Acne medication Toothpaste ingredient
Supercritical Gases		

Chemical Disinfectants		
Chemical	Mode of Action	Example Uses
Carbon dioxide	Penetrates cells, forms carbonic acid, lowers intracellular pH	Food preservation Disinfection of medical devices Disinfection of transplant tissues
Chemical Food Preservatives		
Sorbic acid Benzoic acid Propionic acid Potassium sorbate Sodium benzoate Calcium propionate Sulfur dioxide Nitrites	Decrease pH and inhibit enzymatic function	Preservation of food products
Natural Food Preservatives		
Nisin Natamycin	Inhibition of cell wall synthesis (Nisin)	Preservation of dairy products, meats, and beverages

Key Concepts and Summary

- **Heavy metals**, including mercury, silver, copper, and zinc, have long been used for disinfection and preservation, although some have toxicity and environmental risks associated with them.
- **Halogens**, including chlorine, fluorine, and iodine, are also commonly used for disinfection. Chlorine compounds, including **sodium hypochlorite**, **chloramines**, and **chlorine dioxide**, are commonly used for water disinfection. Iodine, in both **tincture** and **iodophor** forms, is an effective antiseptic.
- **Alcohols**, including ethyl alcohol and isopropyl alcohol, are commonly used antiseptics that act by denaturing proteins and disrupting membranes.
- **Phenolics** are stable, long-acting disinfectants that denature proteins and disrupt membranes. They are commonly found in household cleaners, mouthwashes, and hospital disinfectants, and are also used to preserve harvested crops.
- The phenolic compound **triclosan**, found in antibacterial soaps, plastics, and textiles is technically an antibiotic because of its specific mode of action of inhibiting bacterial fatty-acid synthesis..
- **Surfactants**, including soaps and detergents, lower the surface tension of water to create emulsions that mechanically carry away microbes. Soaps are long-chain fatty acids, whereas detergents are synthetic surfactants.
- **Quaternary ammonium compounds (quats)** are cationic detergents that disrupt membranes. They are used in household cleaners, skin disinfectants, oral rinses, and mouthwashes.
- **Bisbiguanides** disrupt cell membranes, causing cell contents to gel. **Chlorhexidine** and **alexidine** are commonly used for surgical scrubs, for handwashing in clinical settings, and in prescription oral rinses.
- **Alkylating agents** effectively sterilize materials at low temperatures but are carcinogenic and may also irritate tissue. **Glutaraldehyde** and **o-phthalaldehyde** are used as hospital disinfectants but not as antiseptics. **Formaldehyde** is used for the storage of tissue specimens, as an embalming fluid, and in vaccine preparation to inactivate infectious agents. **Ethylene oxide** is a gas sterilant that can permeate heat-sensitive packaged materials, but it is also explosive and carcinogenic.

- **Peroxygens**, including **hydrogen peroxide**, **peracetic acid**, **benzoyl peroxide**, and ozone gas, are strong oxidizing agents that produce free radicals in cells, damaging their macromolecules. They are environmentally safe and are highly effective disinfectants and antiseptics.
- Pressurized carbon dioxide in the form of a **supercritical fluid** easily permeates packaged materials and cells, forming carbonic acid and lowering intracellular pH. Supercritical carbon dioxide is nonreactive, nontoxic, nonflammable, and effective at low temperatures for sterilization of medical devices, implants, and transplanted tissues.
- Chemical preservatives are added to a variety of foods. **Sorbic acid**, **benzoic acid**, **propionic acid**, and their more soluble salts inhibit enzymes or reduce intracellular pH.
- **Sulfites** are used in winemaking and food processing to prevent browning of foods.
- **Nitrites** are used to preserve meats and maintain color, but cooking nitrite-preserved meats may produce carcinogenic nitrosamines.
- **Nisin** and **natamycin** are naturally produced preservatives used in cheeses and meats. Nisin is effective against gram-positive bacteria and natamycin against fungi.

Multiple Choice

Exercise:

Problem:

Which of the following refers to a disinfecting chemical dissolved in alcohol?

- A. iodophor
- B. tincture
- C. phenolic
- D. peroxygen

Solution:

B

Exercise:

Problem:

Which of the following peroxygens is widely used as a household disinfectant, is inexpensive, and breaks down into water and oxygen gas?

- A. hydrogen peroxide
- B. peracetic acid
- C. benzoyl peroxide
- D. ozone

Solution:

A

Exercise:

Problem:

Which of the following chemical food preservatives is used in the wine industry but may cause asthmatic reactions in some individuals?

- A. nitrites
- B. sulfites
- C. propionic acid
- D. benzoic acid

Solution:

B

Exercise:

Problem:

Bleach is an example of which group of chemicals used for disinfection?

- A. heavy metals
- B. halogens
- C. quats
- D. bisbiguanides

Solution:

B

Exercise:**Problem:**

Which chemical disinfectant works by methylating enzymes and nucleic acids and is known for being toxic and carcinogenic?

- A. sorbic acid
- B. triclosan
- C. formaldehyde
- D. hexachlorophene

Solution:

C

Fill in the Blank**Exercise:**

Problem:

Doorknobs and other surfaces in clinical settings are often coated with _____, _____, or _____ to prevent the transmission of microbes.

Solution:

copper, nickel, zinc

True/False**Exercise:**

Problem: Soaps are classified as disinfectants.

Solution:

False

Exercise:**Problem:**

Mercury-based compounds have fallen out of favor for use as preservatives and antiseptics.

Solution:

True

Short Answer**Exercise:**

Problem:

Which solution of ethyl alcohol is more effective at inhibiting microbial growth: a 70% solution or a 100% solution? Why?

Exercise:

Problem:

When might a gas treatment be used to control microbial growth instead of autoclaving? What are some examples?

Exercise:

Problem:

What is the advantage of using an iodophor rather than iodine or an iodine tincture?

Critical Thinking

Exercise:

Problem:

Looking at [\[link\]](#) and reviewing the functional groups in [\[link\]](#), which alkylating agent shown lacks an aldehyde group?

Exercise:

Problem:

Do you think naturally produced antimicrobial products like nisin and natamycin should replace sorbic acid for food preservation? Why or why not?

Exercise:

Problem:

Why is the use of skin disinfecting compounds required for surgical scrubbing and not for everyday handwashing?

Testing the Effectiveness of Antiseptics and Disinfectants

LEARNING OBJECTIVES

- Describe why the phenol coefficient is used
- Compare and contrast the disk-diffusion, use-dilution, and in-use methods for testing the effectiveness of antiseptics, disinfectants, and sterilants

The effectiveness of various chemical disinfectants is reflected in the terms used to describe them. Chemical disinfectants are grouped by the power of their activity, with each category reflecting the types of microbes and viruses its component disinfectants are effective against. High-level germicides have the ability to kill vegetative cells, fungi, viruses, and endospores, leading to sterilization, with extended use. Intermediate-level germicides, as their name suggests, are less effective against endospores and certain viruses, and low-level germicides kill only vegetative cells and certain enveloped viruses, and are ineffective against endospores.

However, several environmental conditions influence the potency of an antimicrobial agent and its effectiveness. For example, length of exposure is particularly important, with longer exposure increasing efficacy. Similarly, the concentration of the chemical agent is also important, with higher concentrations being more effective than lower ones. Temperature, pH, and other factors can also affect the potency of a disinfecting agent.

One method to determine the effectiveness of a chemical agent includes swabbing surfaces before and after use to confirm whether a sterile field was maintained during use. Additional

tests are described in the sections that follow. These tests allow for the maintenance of appropriate disinfection protocols in clinical settings, controlling microbial growth to protect patients, health-care workers, and the community.

Phenol Coefficient

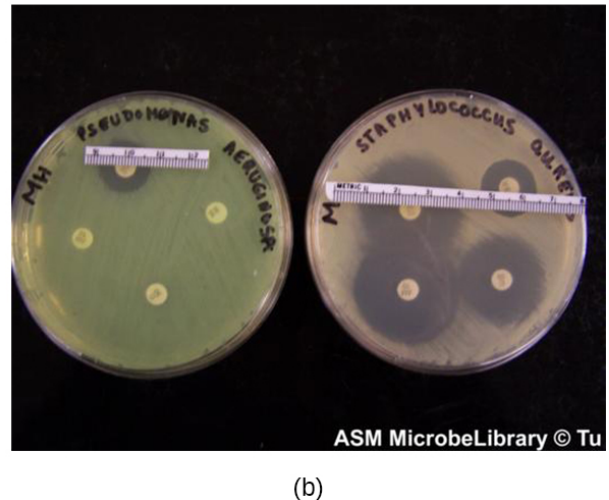
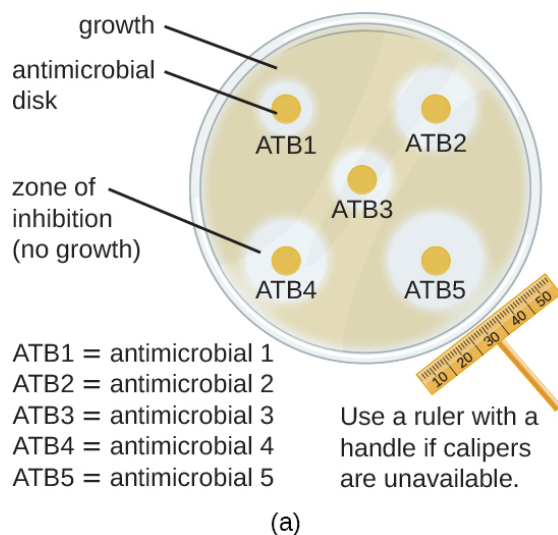
The effectiveness of a disinfectant or antiseptic can be determined in a number of ways. Historically, a chemical agent's effectiveness was often compared with that of phenol, the first chemical agent used by Joseph Lister. In 1903, British chemists Samuel Rideal (1863–1929) and J. T. Ainslie Walker (1868–1930) established a protocol to compare the effectiveness of a variety of chemicals with that of phenol, using as their test organisms *Staphylococcus aureus* (a gram-positive bacterium) and *Salmonella enterica* serovar Typhi (a gram-negative bacterium). They exposed the test bacteria to the antimicrobial chemical solutions diluted in water for 7.5 minutes. They then calculated a phenol coefficient for each chemical for each of the two bacteria tested. A **phenol coefficient** of 1.0 means that the chemical agent has about the same level of effectiveness as phenol. A chemical agent with a phenol coefficient of less than 1.0 is less effective than phenol. An example is formalin, with phenol coefficients of 0.3 (*S. aureus*) and 0.7 (*S. enterica* serovar Typhi). A chemical agent with a phenol coefficient greater than 1.0 is more effective than phenol, such as chloramine, with phenol coefficients of 133 and 100, respectively. Although the phenol coefficient was once a useful measure of effectiveness, it is no longer commonly used because the conditions and organisms used were arbitrarily chosen.

Note:

- What are the differences between the three levels of disinfectant effectiveness?

Disk-Diffusion Method

The **disk-diffusion method** involves applying different chemicals to separate, sterile filter paper disks ([link](#)). The disks are then placed on an agar plate that has been inoculated with the targeted bacterium and the chemicals diffuse out of the disks into the agar where the bacteria have been inoculated. As the “lawn” of bacteria grows, zones of inhibition of microbial growth are observed as clear areas around the disks. Although there are other factors that contribute to the sizes of zones of inhibition (e.g., whether the agent is water soluble and able to diffuse in the agar), larger zones typically correlate to increased inhibition effectiveness of the chemical agent. The diameter across each zone is measured in millimeters.



A disk-diffusion assay is used to determine the effectiveness of chemical agents against a particular microbe. (a) A plate is inoculated with various antimicrobial discs. The zone of inhibition around each disc indicates how effective that antimicrobial is against the particular species being tested. (b) On these plates, four antimicrobial agents are tested for efficacy in killing *Pseudomonas aeruginosa* (left) and

Staphylococcus aureus (right). These antimicrobials are much more effective at killing *S. aureus*, as indicated by the size of the zones of inhibition. (credit b: modification of work by American Society for Microbiology)

Note:

- When comparing the activities of two disinfectants against the same microbe, using the disk-diffusion assay, and assuming both are water soluble and can easily diffuse in the agar, would a more effective disinfectant have a larger zone of inhibition or a smaller one?

Use-Dilution Test

Other methods are also used for measuring the effectiveness of a chemical agent in clinical settings. The **use-dilution test** is commonly used to determine a chemical's disinfection effectiveness on an inanimate surface. For this test, a cylinder of stainless steel is dipped in a culture of the targeted microorganism and then dried. The cylinder is then dipped in solutions of disinfectant at various concentrations for a specified amount of time. Finally, the cylinder is transferred to a new test tube containing fresh sterile medium that does not contain disinfectant, and this test tube is incubated. Bacterial survival is demonstrated by the presence of turbidity in the medium, whereas killing of the target organism on the cylinder by the disinfectant will produce no turbidity.

The Association of Official Agricultural Chemists International (AOAC), a nonprofit group that establishes many protocol standards, has determined that a minimum of 59 of 60 replicates must show no growth in such a test to achieve a passing result, and the results must be repeatable from different batches of disinfectant and when performed on different days. Disinfectant

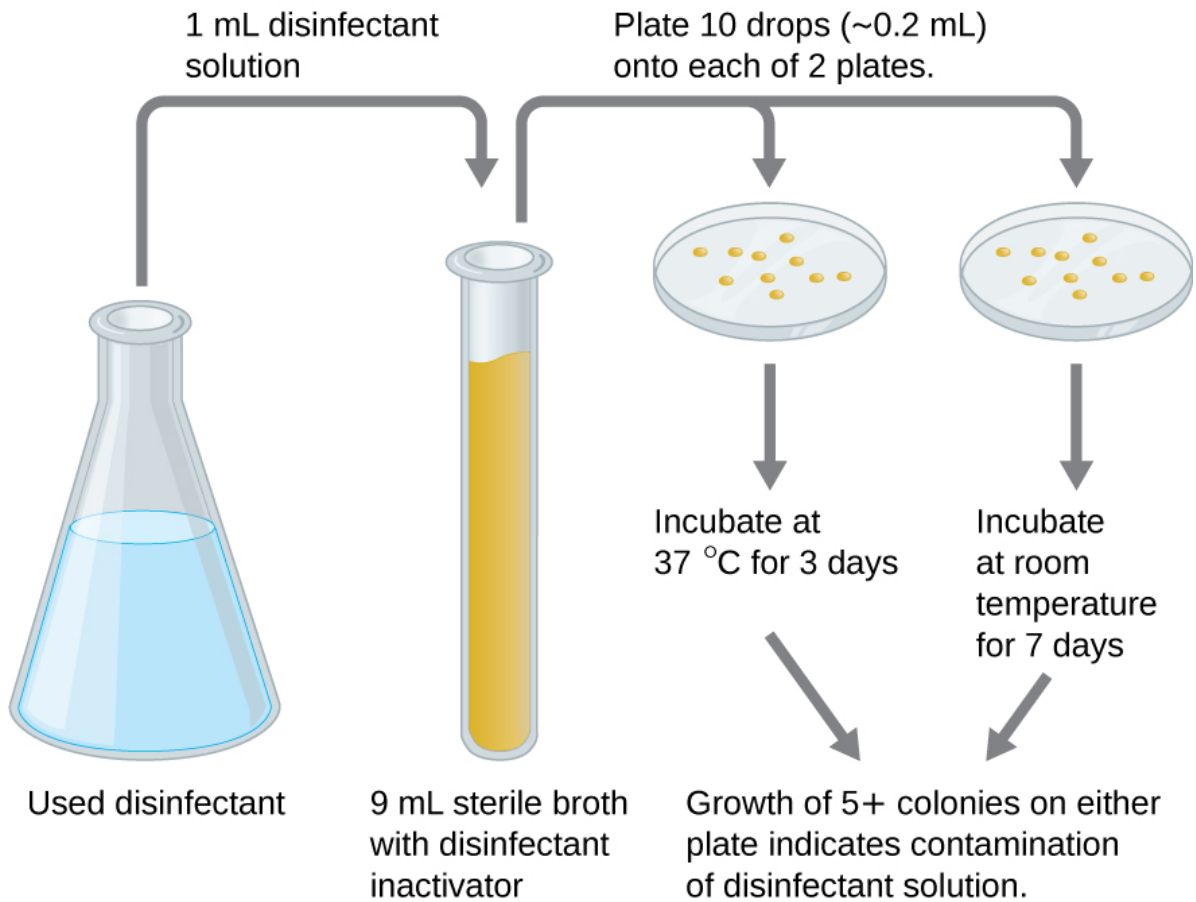
manufacturers perform use-dilution tests to validate the efficacy claims for their products, as designated by the EPA.

Note:

- Is the use-dilution test performed in a clinical setting? Why?

In-Use Test

An **in-use test** can determine whether an actively used solution of disinfectant in a clinical setting is microbially contaminated ([\[link\]](#)). A 1-mL sample of the used disinfectant is diluted into 9 mL of sterile broth medium that also contains a compound to inactivate the disinfectant. Ten drops, totaling approximately 0.2 mL of this mixture, are then inoculated onto each of two agar plates. One plate is incubated at 37 °C for 3 days and the other is incubated at room temperature for 7 days. The plates are monitored for growth of microbial colonies. Growth of five or more colonies on either plate suggests that viable microbial cells existed in the disinfectant solution and that it is contaminated. Such in-use tests monitor the effectiveness of disinfectants in the clinical setting.



Used disinfectant solutions in a clinical setting can be checked with the in-use test for contamination with microbes.

Note:

- What does a positive in-use test indicate?

Note:

Resolution

Despite antibiotic treatment, Roberta's symptoms worsened. She developed pyelonephritis, a severe kidney infection, and was rehospitalized in the intensive care unit (ICU). Her condition continued to deteriorate, and she developed symptoms of septic shock. At this point, her physician ordered a culture from her urine to determine the exact cause of her infection, as well as a drug sensitivity test to determine what antibiotics would be effective against the causative bacterium. The results of this test indicated resistance to a wide range of antibiotics, including the carbapenems, a class of antibiotics that are used as the last resort for many types of bacterial infections. This was an alarming outcome, suggesting that Roberta's infection was caused by a so-called superbug: a bacterial strain that has developed resistance to the majority of commonly used antibiotics. In this case, the causative agent belonged to the carbapenem-resistant Enterobacteriaceae (CRE), a drug-resistant family of bacteria normally found in the digestive system ([\[link\]](#)). When CRE is introduced to other body systems, as might occur through improperly cleaned surgical instruments, catheters, or endoscopes, aggressive infections can occur. CRE infections are notoriously difficult to treat, with a 40%–50% fatality rate. To treat her kidney infection and septic shock, Roberta was treated with dialysis, intravenous fluids, and medications to maintain blood pressure and prevent blood clotting. She was also started on aggressive treatment with intravenous administration of a new drug called tigecycline, which has been successful in treating infections caused by drug-resistant bacteria.

After several weeks in the ICU, Roberta recovered from her CRE infection. However, public health officials soon noticed that Roberta's case was not isolated. Several patients who underwent similar procedures at the same hospital also developed CRE infections, some dying as a result. Ultimately, the source of the infection was traced to the duodenoscopes used in the procedures. Despite the hospital staff meticulously following manufacturer protocols for disinfection, bacteria, including CRE, remained within the instruments and were introduced to patients during procedures.



CRE is an extremely drug-resistant strain of bacteria that is typically associated with nosocomial infections.
(credit: Centers for Disease Control and Prevention)

Go back to the [previous](#) Clinical Focus box.

Note:
Who Is Responsible?

Carbapenem-resistant Enterobacteriaceae infections due to contaminated endoscopes have become a high-profile problem in recent years. Several CRE outbreaks have been traced to endoscopes, including a case at Ronald Reagan UCLA Medical Center in early 2015 in which 179 patients may have been exposed to a contaminated endoscope. Seven of the patients developed infections, and two later died. Several lawsuits have been filed against Olympus, the manufacturer of the endoscopes. Some claim that Olympus did not obtain FDA approval for design changes that may have led to contamination, and others claim that the manufacturer knowingly withheld information from hospitals concerning defects in the endoscopes. Lawsuits like these raise difficult-to-answer questions about liability.

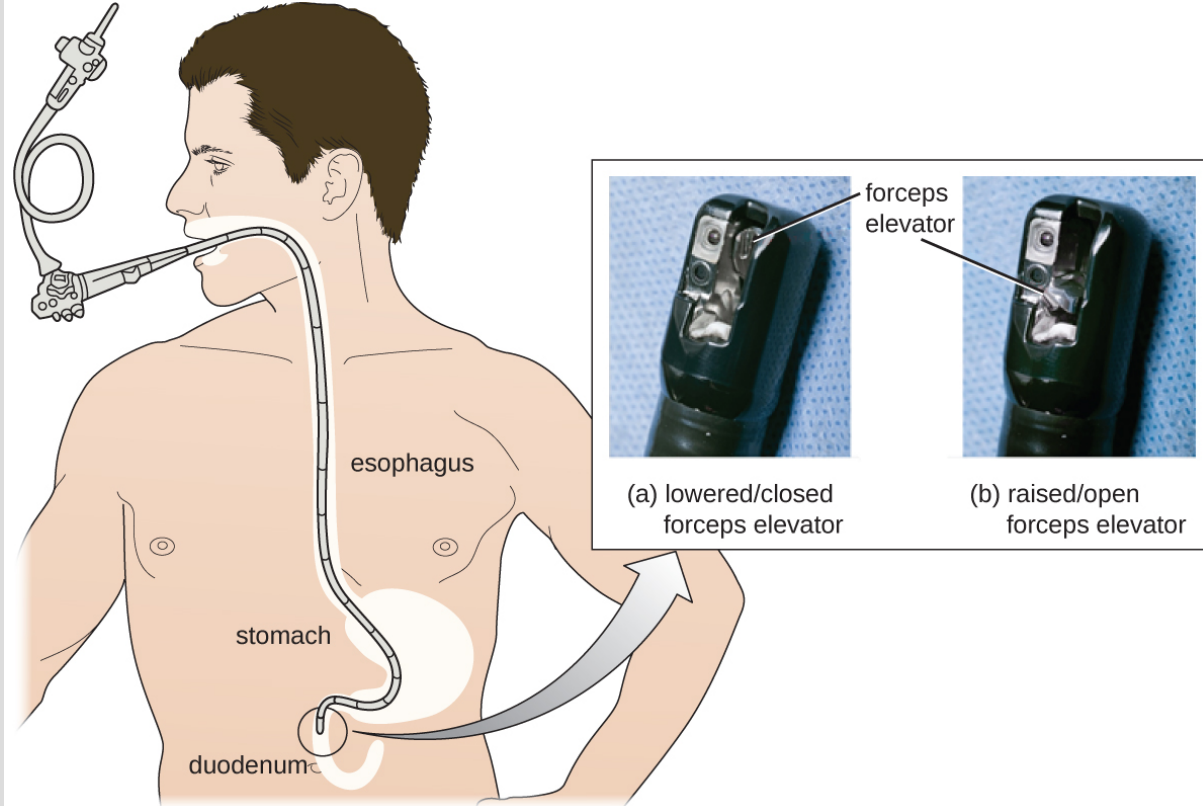
Invasive procedures are inherently risky, but negative outcomes can be minimized by strict adherence to established protocols. Who is responsible, however, when negative outcomes occur due to flawed protocols or faulty equipment? Can hospitals or health-care workers be held liable if they have strictly followed a flawed procedure? Should manufacturers be held liable—and perhaps be driven out of business—if their lifesaving equipment fails or is found defective? What is the government's role in ensuring that use and maintenance of medical equipment and protocols are fail-safe? Protocols for cleaning or sterilizing medical equipment are often developed by government agencies like the FDA, and other groups, like the AOAC, a nonprofit scientific organization that establishes many protocols for standard use globally. These procedures and protocols are then adopted by medical device and equipment manufacturers. Ultimately, the end-users (hospitals and their staff) are responsible for following these procedures and can be held liable if a breach occurs and patients become ill from improperly cleaned equipment.

Unfortunately, protocols are not infallible, and sometimes it takes negative outcomes to reveal their flaws. In 2008, the FDA had approved a disinfection protocol for endoscopes, using glutaraldehyde (at a lower concentration when mixed with phenol), o-phthalaldehyde, hydrogen peroxide, peracetic acid, and a mix of hydrogen peroxide with peracetic acid. However, subsequent CRE outbreaks from endoscope use showed that this protocol alone was inadequate.

As a result of CRE outbreaks, hospitals, manufacturers, and the FDA are investigating solutions. Many hospitals are instituting more rigorous cleaning procedures than those mandated by the FDA. Manufacturers are

looking for ways to redesign duodenoscopes to minimize hard-to-reach crevices where bacteria can escape disinfectants, and the FDA is updating its protocols. In February 2015, the FDA added new recommendations for careful hand cleaning of the duodenoscope elevator mechanism (the location where microbes are most likely to escape disinfection), and issued more careful documentation about quality control of disinfection protocols ([\[link\]](#)).

There is no guarantee that new procedures, protocols, or equipment will completely eliminate the risk for infection associated with endoscopes. Yet these devices are used successfully in 500,000–650,000 procedures annually in the United States, many of them lifesaving. At what point do the risks outweigh the benefits of these devices, and who should be held responsible when negative outcomes occur?



The elevator mechanism in a duodenoscope contains crevices that are difficult to disinfect. Pathogens that survive disinfection protocols can be passed from one patient to another, causing serious infections. (credit “photos”: modification of work by Centers for Disease Control and Prevention)

Key Concepts and Summary

- Chemical disinfectants are grouped by the types of microbes and infectious agents they are effective against. **High-level germicides** kill vegetative cells, fungi, viruses, and endospores, and can ultimately lead to sterilization. **Intermediate-level germicides** cannot kill all viruses and are less effective against endospores. **Low-level germicides** kill vegetative cells and some enveloped viruses, but are ineffective against endospores.
- The effectiveness of a disinfectant is influenced by several factors, including length of exposure, concentration of disinfectant, temperature, and pH.
- Historically, the effectiveness of a chemical disinfectant was compared with that of phenol at killing *Staphylococcus aureus* and *Salmonella enterica* serovar Typhi, and a **phenol coefficient** was calculated.
- The **disk-diffusion method** is used to test the effectiveness of a chemical disinfectant against a particular microbe.
- The **use-dilution test** determines the effectiveness of a disinfectant on a surface. **In-use tests** can determine whether disinfectant solutions are being used correctly in clinical settings.

Multiple Choice

Exercise:

Problem:

Which type of test is used to determine whether disinfectant solutions actively used in a clinical setting are being used correctly?

- A. disk-diffusion assay
- B. phenol coefficient test
- C. in-use test

D. use-dilution test

Solution:

C

Exercise:

Problem:

The effectiveness of chemical disinfectants has historically been compared to that of which of the following?

- A. phenol
 - B. ethyl alcohol
 - C. bleach
 - D. formaldehyde
-

Solution:

A

Exercise:

Problem:

Which of the following refers to a germicide that can kill vegetative cells and certain enveloped viruses but not endospores?

- A. high-level germicide
 - B. intermediate-level germicide
 - C. low-level germicide
 - D. sterilant
-

Solution:

C

Fill in the Blank

Exercise:

Problem:

If a chemical disinfectant is more effective than phenol, then its phenol coefficient would be _____ than 1.0.

Solution:

greater

Exercise:

Problem:

If used for extended periods of time, _____ germicides may lead to sterility.

Solution:

high-level

Exercise:

Problem:

In the disk-diffusion assay, a large zone of inhibition around a disk to which a chemical disinfectant has been applied indicates _____ of the test microbe to the chemical disinfectant.

Solution:

susceptibility or sensitivity

Short Answer

Exercise:

Problem:

Why were chemical disinfectants once commonly compared with phenol?

Exercise:

Problem:

Why is length of exposure to a chemical disinfectant important for its activity?

Critical Thinking

Exercise:

Problem:

What are some advantages of use-dilution and in-use tests compared with the disk-diffusion assay?

Introduction

class="introduction"

First mass
produced in the
1940s,
penicillin was
instrumental in
saving millions
of lives during
World War II
and was
considered a
wonder drug.

[\[footnote\]](#)

Today,
overprescription
of antibiotics
(especially for
childhood
illnesses) has
contributed to
the evolution of
drug-resistant
pathogens.

(credit left:
modification of
work by
Chemical
Heritage
Foundation;
credit right:
modification of
work by U.S.
Department of
Defense)

“Treatment of
War Wounds: A
Historical
Review.”
*Clinical
Orthopaedics
and Related
Research* 467
no. 8
(2009):2168–
2191.



In nature, some microbes produce substances that inhibit or kill other microbes that might otherwise compete for the same resources. Humans have successfully exploited these abilities, using microbes to mass-produce substances that can be used as antimicrobial drugs. Since their discovery, antimicrobial drugs have saved countless lives, and they remain an essential tool for treating and controlling infectious disease. But their widespread and often unnecessary use has had an unintended side effect: the rise of multidrug-resistant microbial strains. In this chapter, we will discuss how antimicrobial drugs work, why microbes develop resistance, and what health professionals can do to encourage responsible use of antimicrobials.

LEARNING OBJECTIVES

- Contrast bacteriostatic versus bactericidal antibacterial activities
- Contrast broad-spectrum drugs versus narrow-spectrum drugs
- Explain the significance of superinfections
- Discuss the significance of dosage and the route of administration of a drug
- Identify factors and variables that can influence the side effects of a drug
- Describe the significance of positive and negative interactions between drugs

Several factors are important in choosing the most appropriate antimicrobial drug therapy, including bacteriostatic versus bactericidal mechanisms, spectrum of activity, dosage and route of administration, the potential for side effects, and the potential interactions between drugs. The following discussion will focus primarily on antibacterial drugs, but the concepts translate to other antimicrobial classes.

Bacteriostatic Versus Bactericidal

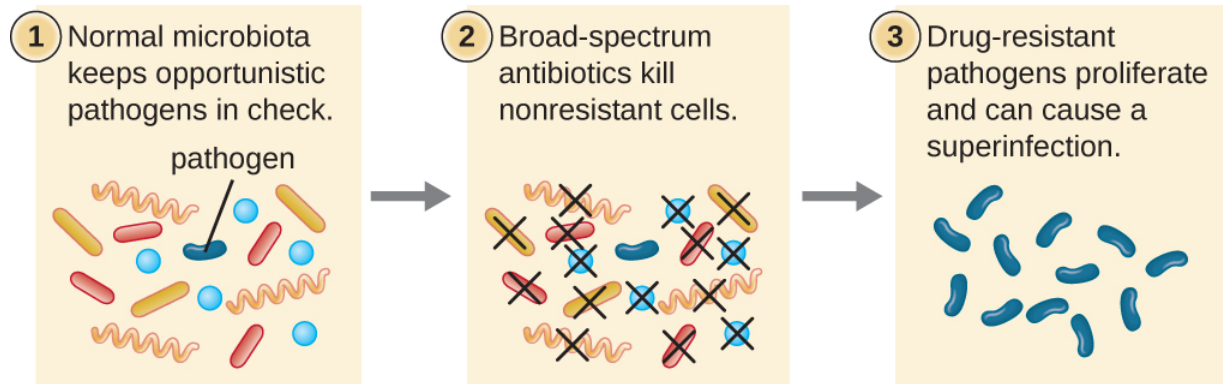
Antibacterial drugs can be either **bacteriostatic** or bactericidal in their interactions with target bacteria. Bacteriostatic drugs cause a reversible inhibition of growth, with bacterial growth restarting after elimination of the drug. By contrast, **bactericidal** drugs kill their target bacteria. The decision of whether to use a bacteriostatic or bactericidal drugs depends on

the type of infection and the immune status of the patient. In a patient with strong immune defenses, bacteriostatic and bactericidal drugs can be effective in achieving clinical cure. However, when a patient is immunocompromised, a bactericidal drug is essential for the successful treatment of infections. Regardless of the immune status of the patient, life-threatening infections such as acute endocarditis require the use of a bactericidal drug.

Spectrum of Activity

The spectrum of activity of an antibacterial drug relates to diversity of targeted bacteria. A **narrow-spectrum antimicrobial** targets only specific subsets of bacterial pathogens. For example, some narrow-spectrum drugs only target gram-positive bacteria, whereas others target only gram-negative bacteria. If the pathogen causing an infection has been identified, it is best to use a narrow-spectrum antimicrobial and minimize collateral damage to the normal microbiota. A **broad-spectrum antimicrobial** targets a wide variety of bacterial pathogens, including both gram-positive and gram-negative species, and is frequently used as empiric therapy to cover a wide range of potential pathogens while waiting on the laboratory identification of the infecting pathogen. Broad-spectrum antimicrobials are also used for polymicrobial infections (mixed infection with multiple bacterial species), or as prophylactic prevention of infections with surgery/invasive procedures. Finally, broad-spectrum antimicrobials may be selected to treat an infection when a narrow-spectrum drug fails because of development of drug resistance by the target pathogen.

The risk associated with using broad-spectrum antimicrobials is that they will also target a broad spectrum of the normal microbiota, increasing the risk of a **superinfection**, a secondary infection in a patient having a preexisting infection. A superinfection develops when the antibacterial intended for the preexisting infection kills the protective microbiota, allowing another pathogen resistant to the antibacterial to proliferate and cause a secondary infection ([\[link\]](#)). Common examples of superinfections that develop as a result of antimicrobial usage include yeast infections (candidiasis) and pseudomembranous colitis caused by *Clostridium difficile*, which can be fatal.



Broad-spectrum antimicrobial use may lead to the development of a superinfection. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- What is a superinfection and how does one arise?

Dosage and Route of Administration

The amount of medication given during a certain time interval is the **dosage**, and it must be determined carefully to ensure that optimum therapeutic drug levels are achieved at the site of infection without causing significant toxicity (side effects) to the patient. Each drug class is associated with a variety of potential side effects, and some of these are described for specific drugs later in this chapter. Despite best efforts to optimize dosing, allergic reactions and other potentially serious side effects do occur. Therefore, the goal is to select the optimum dosage that will minimize the risk of side effects while still achieving clinical cure, and there are important factors to consider when selecting the best dose and dosage interval. For example, in children, dose is based upon the patient's mass.

However, the same is not true for adults and children 12 years of age and older, for which there is typically a single standard dose regardless of the patient's mass. With the great variability in adult body mass, some experts have argued that mass should be considered for all patients when determining appropriate dosage.[\[footnote\]](#) An additional consideration is how drugs are metabolized and eliminated from the body. In general, patients with a history of liver or kidney dysfunction may experience reduced drug metabolism or clearance from the body, resulting in increased drug levels that may lead to toxicity and make them more prone to side effects.

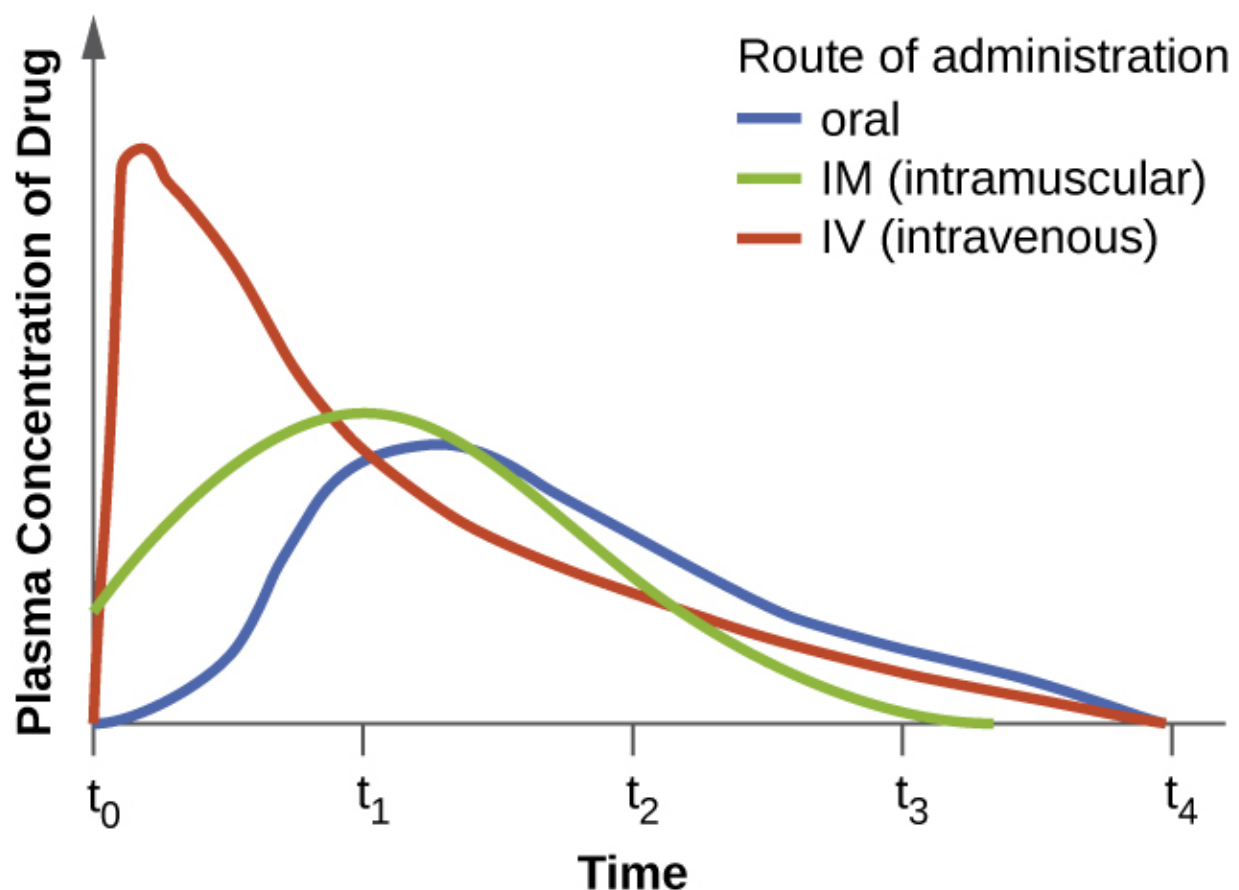
M.E. Falagas, D.E. Karageorgopoulos. "Adjustment of Dosing of Antimicrobial Agents for Bodyweight in Adults." *The Lancet* 375 no. 9710 (2010):248–251.

There are also some factors specific to the drugs themselves that influence appropriate dose and time interval between doses. For example, the half-life, or rate at which 50% of a drug is eliminated from the plasma, can vary significantly between drugs. Some drugs have a short half-life of only 1 hour and must be given multiple times a day, whereas other drugs have half-lives exceeding 12 hours and can be given as a single dose every 24 hours. Although a longer half-life can be considered an advantage for an antibacterial when it comes to convenient dosing intervals, the longer half-life can also be a concern for a drug that has serious side effects because drug levels may remain toxic for a longer time. Last, some drugs are dose dependent, meaning they are more effective when administered in large doses to provide high levels for a short time at the site of infection. Others are time dependent, meaning they are more effective when lower optimum levels are maintained over a longer period of time.

The **route of administration**, the method used to introduce a drug into the body, is also an important consideration for drug therapy. Drugs that can be administered orally are generally preferred because patients can more conveniently take these drugs at home. However, some drugs are not absorbed easily from the gastrointestinal (GI) tract into the bloodstream. These drugs are often useful for treating diseases of the intestinal tract, such as tapeworms treated with niclosamide, or for decontaminating the bowel, as with colistin. Some drugs that are not absorbed easily, such as bacitracin,

polymyxin, and several antifungals, are available as topical preparations for treatment of superficial skin infections. Sometimes, patients may not initially be able to take oral medications because of their illness (e.g., vomiting, intubation for respirator). When this occurs, and when a chosen drug is not absorbed in the GI tract, administration of the drug by a parenteral route (intravenous or intramuscular injection) is preferred and typically is performed in health-care settings. For most drugs, the plasma levels achieved by intravenous administration is substantially higher than levels achieved by oral or intramuscular administration, and this can also be an important consideration when choosing the route of administration for treating an infection ([link](#)).

Plasma Concentration of Drug as a Function of Response Time



On this graph, t_0 represents the time at which a drug dose is administered. The curves illustrate how plasma concentration of the drug changes over specific intervals of time (t_1 through t_4). As the graph shows, when a drug is administered intravenously, the concentration peaks very quickly and then gradually decreases. When drugs are administered orally or intramuscularly, it takes longer for the concentration to reach its peak.

Note:

- List five factors to consider when determining the dosage of a drug.
- Name some typical side effects associated with drugs and identify some factors that might contribute to these side effects.

Drug Interactions

For the optimum treatment of some infections, two antibacterial drugs may be administered together to provide a synergistic interaction that is better than the efficacy of either drug alone. A classic example of synergistic combinations is trimethoprim and sulfamethoxazole (Bactrim). Individually, these two drugs provide only bacteriostatic inhibition of bacterial growth, but combined, the drugs are bactericidal.

Whereas synergistic drug interactions provide a benefit to the patient, antagonistic interactions produce harmful effects. Antagonism can occur between two antimicrobials or between antimicrobials and nonantimicrobials being used to treat other conditions. The effects vary depending on the drugs involved, but antagonistic interactions may cause loss of drug activity, decreased therapeutic levels due to increased metabolism and elimination, or increased potential for toxicity due to decreased metabolism and elimination. As an example, some antibacterials

are absorbed most effectively from the acidic environment of the stomach. If a patient takes antacids, however, this increases the pH of the stomach and negatively impacts the absorption of these antimicrobials, decreasing their effectiveness in treating an infection. Studies have also shown an association between use of some antimicrobials and failure of oral contraceptives.[\[footnote\]](#)

B.D. Dickinson et al. “Drug Interactions between Oral Contraceptives and Antibiotics.” *Obstetrics & Gynecology* 98, no. 5 (2001):853–860.

Note:

- Explain the difference between synergistic and antagonistic drug interactions.

Note:

Resistance Police

In the United States and many other countries, most antimicrobial drugs are self-administered by patients at home. Unfortunately, many patients stop taking antimicrobials once their symptoms dissipate and they feel better. If a 10-day course of treatment is prescribed, many patients only take the drug for 5 or 6 days, unaware of the negative consequences of not completing the full course of treatment. A shorter course of treatment not only fails to kill the target organisms to expected levels, it also selects for drug-resistant variants within the target population and within the patient’s microbiota.

Patients’ nonadherence especially amplifies drug resistance when the recommended course of treatment is long. Treatment for tuberculosis (TB) is a case in point, with the recommended treatment lasting from 6 months to a year. The CDC estimates that about one-third of the world’s population is infected with TB, most living in underdeveloped or underserved regions where antimicrobial drugs are available over the counter. In such countries, there may be even lower rates of adherence than in developed areas. Nonadherence leads to antibiotic resistance and more difficulty in

controlling pathogens. As a direct result, the emergence of multidrug-resistant and extensively drug-resistant strains of TB is becoming a huge problem.

Overprescription of antimicrobials also contributes to antibiotic resistance. Patients often demand antibiotics for diseases that do not require them, like viral colds and ear infections. Pharmaceutical companies aggressively market drugs to physicians and clinics, making it easy for them to give free samples to patients, and some pharmacies even offer certain antibiotics free to low-income patients with a prescription.

In recent years, various initiatives have aimed to educate parents and clinicians about the judicious use of antibiotics. However, a recent study showed that, between 2000 and 2013, the parental expectation for antimicrobial prescriptions for children actually increased ([\[link\]](#)).

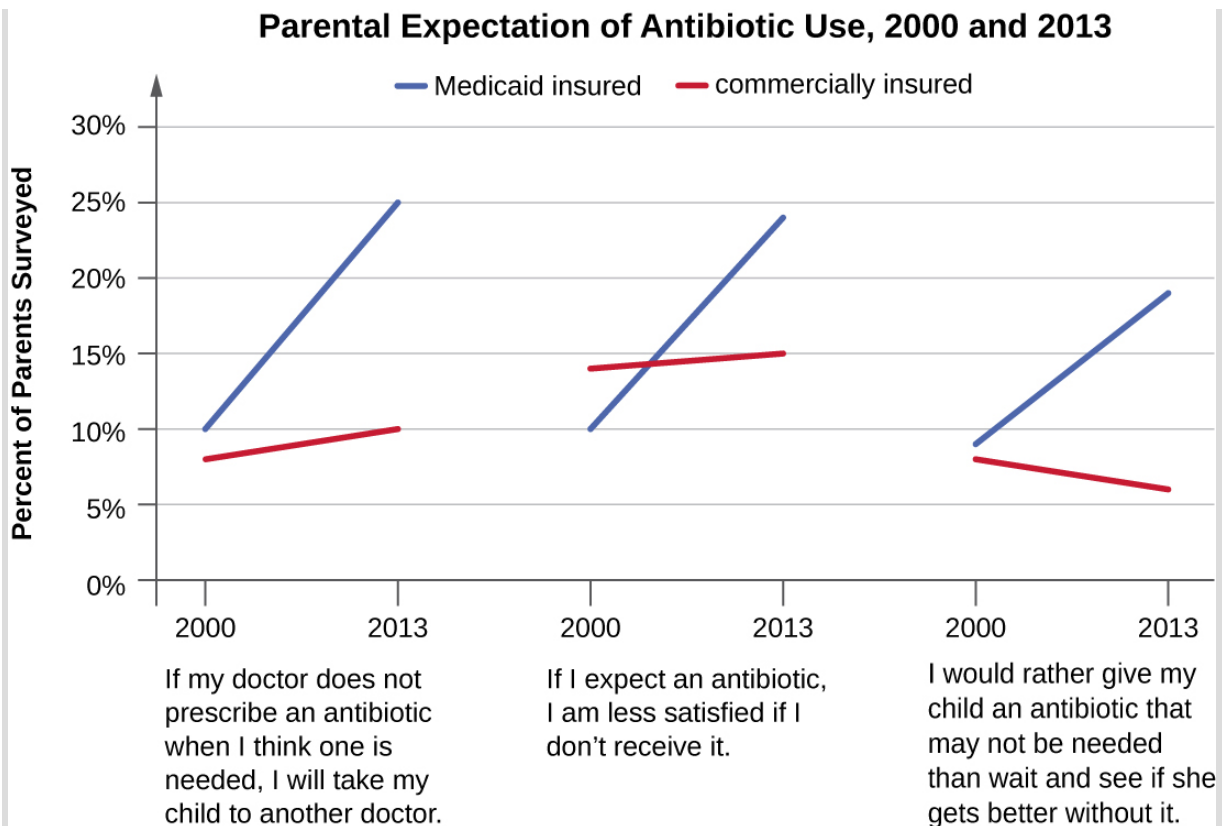
One possible solution is a regimen called directly observed therapy (DOT), which involves the supervised administration of medications to patients. Patients are either required to visit a health-care facility to receive their medications, or health-care providers must administer medication in patients' homes or another designated location. DOT has been implemented in many cases for the treatment of TB and has been shown to be effective; indeed, DOT is an integral part of WHO's global strategy for eradicating TB. [\[footnote\]](#), [\[footnote\]](#) But is this a practical strategy for all antibiotics? Would patients taking penicillin, for example, be more or less likely to adhere to the full course of treatment if they had to travel to a health-care facility for each dose? And who would pay for the increased cost associated with DOT? When it comes to overprescription, should someone be policing physicians or drug companies to enforce best practices? What group should assume this responsibility, and what penalties would be effective in discouraging overprescription?

Centers for Disease Control and Prevention. "Tuberculosis (TB)."

<http://www.cdc.gov/tb/education/ssmodules/module9/ss9reading2.htm>.

Accessed June 2, 2016.

World Health Organization. "Tuberculosis (TB): The Five Elements of DOTS." <http://www.who.int/tb/dots/whatisdots/en/>. Accessed June 2, 2016.



This graph indicates trends in parental expectations related to prescription of antibiotics based on a recent study.[\[footnote\]](#) Among parents of Medicaid-insured children, there was a clear upward trend in parental expectations for prescription antibiotics. Expectations were relatively stable (and lesser) among parents whose children were commercially insured, suggesting that these parents were somewhat better informed than those with Medicaid-insured children.

Vaz, L.E., et al. "Prevalence of Parental Misconceptions About Antibiotic Use." *Pediatrics* 136 no.2 (August 2015). DOI: 10.1542/peds.2015-0883.

Key Concepts and Summary

- Antimicrobial drugs can be **bacteriostatic** or **bactericidal**, and these characteristics are important considerations when selecting the most appropriate drug.
- The use of **narrow-spectrum** antimicrobial drugs is preferred in many cases to avoid **superinfection** and the development of antimicrobial resistance.
- **Broad-spectrum** antimicrobial use is warranted for serious systemic infections when there is no time to determine the causative agent, when narrow-spectrum antimicrobials fail, or for the treatment or prevention of infections with multiple types of microbes.
- The **dosage** and **route of administration** are important considerations when selecting an antimicrobial to treat an infection. Other considerations include the patient's age, mass, ability to take oral medications, liver and kidney function, and possible interactions with other drugs the patient may be taking.

Multiple Choice

Exercise:

Problem:

Which of the following combinations would most likely contribute to the development of a superinfection?

- A. long-term use of narrow-spectrum antimicrobials
- B. long-term use of broad-spectrum antimicrobials
- C. short-term use of narrow-spectrum antimicrobials
- D. short-term use of broad-spectrum antimicrobials

Solution:

B

Exercise:

Problem:

Which of the following routes of administration would be appropriate and convenient for home administration of an antimicrobial to treat a systemic infection?

- A. oral
- B. intravenous
- C. topical
- D. parenteral

Solution:

A

Exercise:**Problem:**

Which clinical situation would be appropriate for treatment with a narrow-spectrum antimicrobial drug?

- A. treatment of a polymicrobial mixed infection in the intestine
- B. prophylaxis against infection after a surgical procedure
- C. treatment of strep throat caused by culture identified *Streptococcus pyogenes*
- D. empiric therapy of pneumonia while waiting for culture results

Solution:

C

Fill in the Blank**Exercise:**

Problem:

The bacterium known for causing pseudomembranous colitis, a potentially deadly superinfection, is _____.

Solution:

Clostridium difficile

True/False**Exercise:****Problem:**

Narrow-spectrum antimicrobials are commonly used for prophylaxis following surgery.

Solution:

False

Short Answer**Exercise:****Problem:**

When prescribing antibiotics, what aspects of the patient's health history should the clinician ask about and why?

Exercise:**Problem:**

When is using a broad-spectrum antimicrobial drug warranted?

Critical Thinking

Exercise:

Problem:

Why are yeast infections a common type of superinfection that results from long-term use of broad-spectrum antimicrobials?

Exercise:

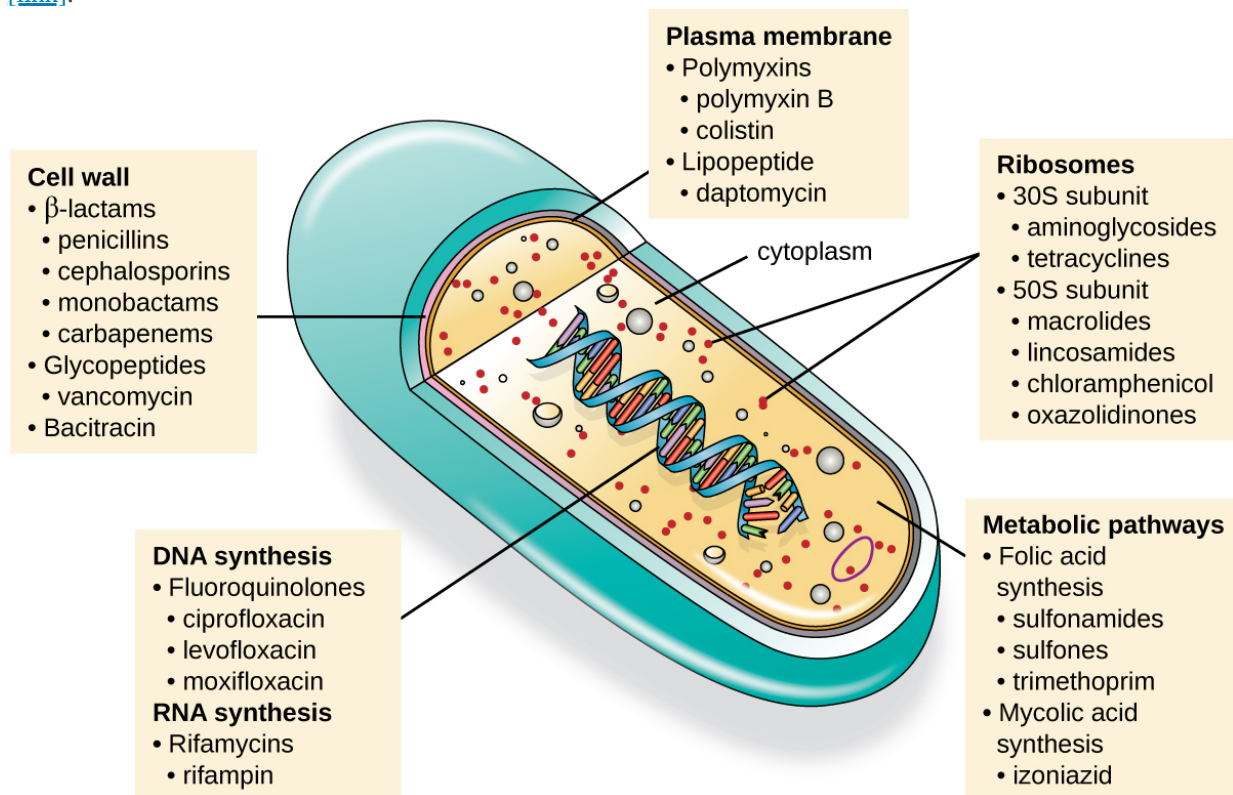
Problem:

Too often patients will stop taking antimicrobial drugs before the prescription is finished. What are factors that cause a patient to stop too soon, and what negative impacts could this have?

LEARNING OBJECTIVE

- Describe the mechanisms of action associated with drugs that inhibit cell wall biosynthesis, protein synthesis, membrane function, nucleic acid synthesis, and metabolic pathways

An important quality for an antimicrobial drug is **selective toxicity**, meaning that it selectively kills or inhibits the growth of microbial targets while causing minimal or no harm to the host. Most antimicrobial drugs currently in clinical use are antibacterial because the prokaryotic cell provides a greater variety of unique targets for selective toxicity, in comparison to fungi, parasites, and viruses. Each class of antibacterial drugs has a unique **mode of action** (the way in which a drug affects microbes at the cellular level), and these are summarized in [\[link\]](#) and [\[link\]](#).



There are several classes of antibacterial compounds that are typically classified based on their bacterial target.

Common Antibacterial Drugs by Mode of Action		
Mode of Action	Target	Drug Class
Inhibit cell wall biosynthesis	Penicillin-binding proteins	β -lactams: penicillins, cephalosporins, monobactams, carbapenems
	Peptidoglycan subunits	Glycopeptides
	Peptidoglycan subunit transport	Bacitracin
Inhibit biosynthesis of proteins	30S ribosomal subunit	Aminoglycosides, tetracyclines
	50S ribosomal subunit	Macrolides, lincosamides, chloramphenicol, oxazolidinones
Disrupt membranes	Lipopolysaccharide, inner and outer membranes	Polymyxin B, colistin, daptomycin
Inhibit nucleic acid synthesis	RNA	Rifamycin
	DNA	Fluoroquinolones
Antimetabolites	Folic acid synthesis enzyme	Sulfonamides, trimethoprim
	Mycolic acid synthesis enzyme	Isonicotinic acid hydrazide
Mycobacterial adenosine triphosphate (ATP) synthase inhibitor	Mycobacterial ATP synthase	Diarylquinoline

Inhibitors of Cell Wall Biosynthesis

Several different classes of antibacterials block steps in the biosynthesis of peptidoglycan, making cells more susceptible to osmotic lysis ([\[link\]](#)). Therefore, antibacterials that target cell wall biosynthesis are bactericidal in their action. Because human cells do not make peptidoglycan, this mode of action is an excellent example of selective toxicity.

Penicillin, the first antibiotic discovered, is one of several antibacterials within a class called **β -lactams**. This group of compounds includes the penicillins, cephalosporins, monobactams, and carbapenems, and is characterized by the presence of a β -lactam ring found within the central structure of the drug molecule ([\[link\]](#)). The β -lactam antibacterials block the crosslinking of peptide chains during the biosynthesis of new peptidoglycan in the bacterial cell wall. They are able to block this process because the β -lactam structure is similar to the structure of the peptidoglycan subunit component that is recognized by the crosslinking transpeptidase enzyme, also known as a penicillin-binding protein (PBP). Although the β -lactam ring must remain unchanged for these drugs to retain their antibacterial activity, strategic chemical changes to the R groups have allowed for development of a wide variety of semisynthetic β -lactam drugs with increased potency, expanded spectrum of activity, and longer half-lives for better dosing, among other characteristics.

Penicillin G and penicillin V are natural antibiotics from fungi and are primarily active against gram-positive bacterial pathogens, and a few gram-negative bacterial pathogens such as *Pasteurella multocida*. [\[link\]](#)

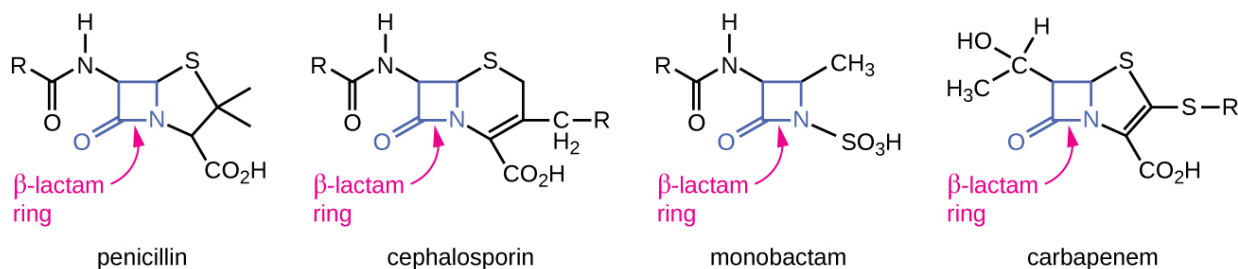
summarizes the semisynthetic development of some of the penicillins. Adding an amino group (-NH₂) to penicillin G created the aminopenicillins (i.e., ampicillin and amoxicillin) that have increased spectrum of activity against more gram-negative pathogens. Furthermore, the addition of a hydroxyl group (-OH) to amoxicillin increased acid stability, which allows for improved oral absorption. Methicillin is a semisynthetic penicillin that was developed to address the spread of enzymes (penicillinases) that were inactivating the other penicillins. Changing the R group of penicillin G to the more bulky dimethoxyphenyl group provided protection of the β -lactam ring from enzymatic destruction by penicillinases, giving us the first penicillinase-resistant penicillin.

Similar to the penicillins, **cephalosporins** contain a β -lactam ring ([link](#)) and block the transpeptidase activity of penicillin-binding proteins. However, the β -lactam ring of cephalosporins is fused to a six-member ring, rather than the five-member ring found in penicillins. This chemical difference provides cephalosporins with an increased resistance to enzymatic inactivation by **β -lactamases**. The drug cephalosporin C was originally isolated from the fungus *Cephalosporium acremonium* in the 1950s and has a similar spectrum of activity to that of penicillin against gram-positive bacteria but is active against more gram-negative bacteria than penicillin. Another important structural difference is that cephalosporin C possesses two R groups, compared with just one R group for penicillin, and this provides for greater diversity in chemical alterations and development of semisynthetic cephalosporins. The family of semisynthetic cephalosporins is much larger than the penicillins, and these drugs have been classified into generations based primarily on their spectrum of activity, increasing in spectrum from the narrow-spectrum, first-generation cephalosporins to the broad-spectrum, fourth-generation cephalosporins. A new fifth-generation cephalosporin has been developed that is active against methicillin-resistant *Staphylococcus aureus* (MRSA).

The carbapenems and monobactams also have a β -lactam ring as part of their core structure, and they inhibit the transpeptidase activity of penicillin-binding proteins. The only monobactam used clinically is aztreonam. It is a narrow-spectrum antibacterial with activity only against gram-negative bacteria. In contrast, the carbapenem family includes a variety of semisynthetic drugs (imipenem, meropenem, and doripenem) that provide very broad-spectrum activity against gram-positive and gram-negative bacterial pathogens.

The drug **vancomycin**, a member of a class of compounds called the **glycopeptides**, was discovered in the 1950s as a natural antibiotic from the actinomycete *Amycolatopsis orientalis*. Similar to the β -lactams, vancomycin inhibits cell wall biosynthesis and is bactericidal. However, in contrast to the β -lactams, the structure of vancomycin is not similar to that of cell-wall peptidoglycan subunits and does not directly inactivate penicillin-binding proteins. Rather, vancomycin is a very large, complex molecule that binds to the end of the peptide chain of cell wall precursors, creating a structural blockage that prevents the cell wall subunits from being incorporated into the growing N-acetylglucosamine and N-acetylmuramic acid (NAM-NAG) backbone of the peptidoglycan structure (transglycosylation). Vancomycin also structurally blocks transpeptidation. Vancomycin is bactericidal against gram-positive bacterial pathogens, but it is not active against gram-negative bacteria because of its inability to penetrate the protective outer membrane.

The drug **bacitracin** consists of a group of structurally similar peptide antibiotics originally isolated from *Bacillus subtilis*. Bacitracin blocks the activity of a specific cell-membrane molecule that is responsible for the movement of peptidoglycan precursors from the cytoplasm to the exterior of the cell, ultimately preventing their incorporation into the cell wall. Bacitracin is effective against a wide range of bacteria, including gram-positive organisms found on the skin, such as *Staphylococcus* and *Streptococcus*. Although it may be administered orally or intramuscularly in some circumstances, bacitracin has been shown to be nephrotoxic (damaging to the kidneys). Therefore, it is more commonly combined with neomycin and polymyxin in topical ointments such as Neosporin.



R group					
Drug name	penicillin G	penicillin V	ampicillin	amoxicillin	methicillin
Spectrum of activity	G+ and a few G-	similar to penicillin G	G+ and more G- than penicillin	similar to ampicillin	G+ only, including β-lactamase producers
Route of administration	parenteral	oral	parenteral and oral	oral (better than ampicillin)	parenteral

Penicillins, cephalosporins, monobactams, and carbapenems all contain a β-lactam ring, the site of attack by inactivating β-lactamase enzymes. Although they all share the same nucleus, various penicillins differ from each other in the structure of their R groups. Chemical changes to the R groups provided increased spectrum of activity, acid stability, and resistance to β-lactamase degradation.

Drugs that Inhibit Bacterial Cell Wall Synthesis				
Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
Interact directly with PBPs and inhibit transpeptidase activity	Penicillins	Penicillin G, penicillin V	Natural	Narrow-spectrum against gram-positive and a few gram-negative bacteria

Drugs that Inhibit Bacterial Cell Wall Synthesis				
Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
		Ampicillin, amoxicillin	Semisynthetic	Narrow-spectrum against gram-positive bacteria but with increased gram-negative spectrum
		Methicillin	Semisynthetic	Narrow-spectrum against gram-positive bacteria only, including strains producing penicillinase
	Cephalosporins	Cephalosporin C	Natural	Narrow-spectrum similar to penicillin but with increased gram-negative spectrum
		First-generation cephalosporins	Semisynthetic	Narrow-spectrum similar to cephalosporin C
		Second-generation cephalosporins	Semisynthetic	Narrow-spectrum but with increased gram-negative spectrum compared with first generation

Drugs that Inhibit Bacterial Cell Wall Synthesis				
Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
		Third- and fourth-generation cephalosporins	Semisynthetic	Broad-spectrum against gram-positive and gram-negative bacteria, including some β -lactamase producers
		Fifth-generation cephalosporins	Semisynthetic	Broad-spectrum against gram-positive and gram-negative bacteria, including MRSA
	Monobactams	Aztreonam	Semisynthetic	Narrow-spectrum against gram-negative bacteria, including some β -lactamase producers
	Carbapenems	Imipenem, meropenem, doripenem	Semisynthetic	Broadest spectrum of the β -lactams against gram-positive and gram-negative bacteria, including many β -lactamase producers

Drugs that Inhibit Bacterial Cell Wall Synthesis				
Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
Large molecules that bind to the peptide chain of peptidoglycan subunits, blocking transglycosylation and transpeptidation	Glycopeptides	Vancomycin	Natural	Narrow spectrum against gram-positive bacteria only, including multidrug-resistant strains
Block transport of peptidoglycan subunits across cytoplasmic membrane	Bacitracin	Bacitracin	Natural	Broad-spectrum against gram-positive and gram-negative bacteria

Note:

- Describe the mode of action of β -lactams.

Inhibitors of Protein Biosynthesis

The cytoplasmic ribosomes found in animal cells (80S) are structurally distinct from those found in bacterial cells (70S), making protein biosynthesis a good selective target for antibacterial drugs. Several types of protein biosynthesis inhibitors are discussed in this section and are summarized in [\[link\]](#).

Protein Synthesis Inhibitors That Bind the 30S Subunit

Aminoglycosides are large, highly polar antibacterial drugs that bind to the 30S subunit of bacterial ribosomes, impairing the proofreading ability of the ribosomal complex. This impairment causes mismatches between codons and anticodons, resulting in the production of proteins with incorrect amino acids and shortened proteins that insert into the cytoplasmic membrane. Disruption of the cytoplasmic membrane by the faulty proteins kills the bacterial cells. The **aminoglycosides**, which include drugs such as streptomycin, gentamicin, neomycin, and kanamycin, are potent broad-spectrum antibacterials. However, aminoglycosides have been shown to be nephrotoxic (damaging to kidney), neurotoxic (damaging to the nervous system), and ototoxic (damaging to the ear).

Another class of antibacterial compounds that bind to the 30S subunit is the **tetracyclines**. In contrast to aminoglycosides, these drugs are bacteriostatic and inhibit protein synthesis by blocking the association of tRNAs with the ribosome during translation. Naturally occurring tetracyclines produced by various strains of *Streptomyces* were first discovered in the 1940s, and several semisynthetic tetracyclines, including doxycycline and tigecycline have also been produced. Although the tetracyclines are broad spectrum in their coverage of bacterial pathogens,

side effects that can limit their use include phototoxicity, permanent discoloration of developing teeth, and liver toxicity with high doses or in patients with kidney impairment.

Protein Synthesis Inhibitors That Bind the 50S Subunit

There are several classes of antibacterial drugs that work through binding to the 50S subunit of bacterial ribosomes. The macrolide antibacterial drugs have a large, complex ring structure and are part of a larger class of naturally produced secondary metabolites called polyketides, complex compounds produced in a stepwise fashion through the repeated addition of two-carbon units by a mechanism similar to that used for fatty acid synthesis. Macrolides are broad-spectrum, bacteriostatic drugs that block elongation of proteins by inhibiting peptide bond formation between specific combinations of amino acids. The first macrolide was **erythromycin**. It was isolated in 1952 from *Streptomyces erythreus* and prevents translocation. Semisynthetic macrolides include azithromycin and telithromycin. Compared with erythromycin, **azithromycin** has a broader spectrum of activity, fewer side effects, and a significantly longer half-life (1.5 hours for erythromycin versus 68 hours for azithromycin) that allows for once-daily dosing and a short 3-day course of therapy (i.e., Zpac formulation) for most infections. Telithromycin is the first semisynthetic within the class known as ketolides. Although telithromycin shows increased potency and activity against macrolide-resistant pathogens, the US Food and Drug Administration (FDA) has limited its use to treatment of community-acquired pneumonia and requires the strongest “black box warning” label for the drug because of serious hepatotoxicity.

The **lincosamides** include the naturally produced **lincomycin** and semisynthetic **clindamycin**. Although structurally distinct from macrolides, lincosamides are similar in their mode of action to the macrolides through binding to the 50S ribosomal subunit and preventing peptide bond formation. Lincosamides are particularly active against streptococcal and staphylococcal infections.

The drug **chloramphenicol** represents yet another structurally distinct class of antibacterials that also bind to the 50S ribosome, inhibiting peptide bond formation. Chloramphenicol, produced by *Streptomyces venezuelae*, was discovered in 1947; in 1949, it became the first broad-spectrum antibiotic that was approved by the FDA. Although it is a natural antibiotic, it is also easily synthesized and was the first antibacterial drug synthetically mass produced. As a result of its mass production, broad-spectrum coverage, and ability to penetrate into tissues efficiently, chloramphenicol was historically used to treat a wide range of infections, from meningitis to typhoid fever to conjunctivitis. Unfortunately, serious side effects, such as lethal gray baby syndrome, and suppression of bone marrow production, have limited its clinical role. Chloramphenicol also causes anemia in two different ways. One mechanism involves the targeting of mitochondrial ribosomes within hematopoietic stem cells, causing a reversible, dose-dependent suppression of blood cell production. Once chloramphenicol dosing is discontinued, blood cell production returns to normal. This mechanism highlights the similarity between 70S ribosomes of bacteria and the 70S ribosomes within our mitochondria. The second mechanism of anemia is idiosyncratic (i.e., the mechanism is not understood), and involves an irreversible lethal loss of blood cell production known as aplastic anemia. This mechanism of aplastic anemia is not dose dependent and can develop after therapy has stopped. Because of toxicity concerns, chloramphenicol usage in humans is now rare in the United States and is limited to severe infections unable to be treated by less toxic antibiotics. Because its side effects are much less severe in animals, it is used in veterinary medicine.

The **oxazolidinones**, including linezolid, are a new broad-spectrum class of synthetic protein synthesis inhibitors that bind to the 50S ribosomal subunit of both gram-positive and gram-negative bacteria. However, their mechanism of action seems somewhat different from that of the other 50S subunit-binding protein synthesis inhibitors already discussed. Instead, they seem to interfere with formation of the initiation complex (association of the 50S subunit, 30S subunit, and other factors) for translation, and they prevent translocation of the growing protein from the ribosomal A site to the P site. [\[link\]](#) summarizes the protein synthesis inhibitors.

Major classes of protein synthesis–inhibiting antibacterials

Chloramphenicol, macrolides, and lincosamides

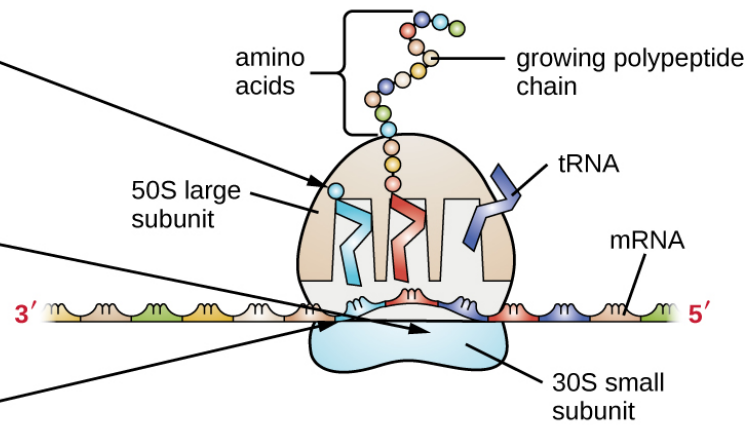
- Bind to the 50S ribosomal subunit
- Prevent peptide bond formation
- Stop protein synthesis

Aminoglycosides

- Bind to the 30S ribosomal subunit
- Impair proofreading, resulting in production of faulty proteins

Tetracyclines

- Bind to the 30S ribosomal subunit
- Block the binding of tRNAs, thereby inhibiting protein synthesis



The major classes of protein synthesis inhibitors target the 30S or 50S subunits of cytoplasmic ribosomes.

Drugs That Inhibit Bacterial Protein Synthesis					
Molecular Target	Mechanism of Action	Drug Class	Specific Drugs	Bacteriostatic or Bactericidal	Spectrum of Activity
30S subunit	Causes mismatches between codons and anticodons, leading to faulty proteins that insert into and disrupt cytoplasmic membrane	Aminoglycosides	Streptomycin, gentamicin, neomycin, kanamycin	Bactericidal	Broad spectrum
	Blocks association of tRNAs with ribosome	Tetracyclines	Tetracycline, doxycycline, tigecycline	Bacteriostatic	Broad spectrum

Drugs That Inhibit Bacterial Protein Synthesis					
Molecular Target	Mechanism of Action	Drug Class	Specific Drugs	Bacteriostatic or Bactericidal	Spectrum of Activity
50S subunit	Blocks peptide bond formation between amino acids	Macrolides	Erythromycin, azithromycin, telithromycin	Bacteriostatic	Broad spectrum
		Lincosamides	Lincomycin, clindamycin	Bacteriostatic	Narrow spectrum
		Not applicable	Chloramphenicol	Bacteriostatic	Broad spectrum
	Interferes with the formation of the initiation complex between 50S and 30S subunits and other factors.	Oxazolidinones	Linezolid	Bacteriostatic	Broad spectrum

Note:

- Compare and contrast the different types of protein synthesis inhibitors.

Inhibitors of Membrane Function

A small group of antibacterials target the bacterial membrane as their mode of action ([\[link\]](#)). The **polymyxins** are natural polypeptide antibiotics that were first discovered in 1947 as products of *Bacillus polymyxa*; only polymyxin B and polymyxin E (**colistin**) have been used clinically. They are lipophilic with detergent-like properties and interact with the lipopolysaccharide component of the outer membrane of gram-negative bacteria, ultimately disrupting both their outer and inner membranes and killing the bacterial cells. Unfortunately, the membrane-targeting mechanism is not a selective toxicity, and these drugs also target and damage the membrane of cells in the kidney and nervous system when administered systemically. Because of these serious side effects and their poor absorption from the digestive tract, polymyxin B is used in over-the-counter topical antibiotic ointments (e.g., Neosporin), and oral colistin was historically used only for bowel decontamination to prevent infections originating from bowel microbes in immunocompromised patients or for those undergoing certain abdominal surgeries. However, the emergence and spread of multidrug-resistant pathogens has led to increased use of intravenous colistin in hospitals, often as a drug of last resort to treat serious infections. The antibacterial **daptomycin** is a cyclic lipopeptide produced by *Streptomyces roseosporus* that seems to work like the polymyxins, inserting in the bacterial cell membrane and disrupting it. However, in contrast to polymyxin B and colistin, which target only gram-negative bacteria, daptomycin specifically targets gram-positive bacteria. It is

typically administered intravenously and seems to be well tolerated, showing reversible toxicity in skeletal muscles.

Drugs That Inhibit Bacterial Membrane Function				
Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity	Clinical Use
Interacts with lipopolysaccharide in the outer membrane of gram-negative bacteria, killing the cell through the eventual disruption of the outer membrane and cytoplasmic membrane	Polymyxins	Polymyxin B	Narrow spectrum against gram-negative bacteria, including multidrug-resistant strains	Topical preparations to prevent infections in wounds
		Polymyxin E (colistin)	Narrow spectrum against gram-negative bacteria, including multidrug-resistant strains	Oral dosing to decontaminate bowels to prevent infections in immunocompromised patients or patients undergoing invasive surgery/procedures.
				Intravenous dosing to treat serious systemic infections caused by multidrug-resistant pathogens
Inserts into the cytoplasmic membrane of gram-positive bacteria, disrupting the membrane and killing the cell	Lipopeptide	Daptomycin	Narrow spectrum against gram-positive bacteria, including multidrug-resistant strains	Complicated skin and skin-structure infections and bacteremia caused by gram-positive pathogens, including MRSA

Note:

- How do polymyxins inhibit membrane function?

Inhibitors of Nucleic Acid Synthesis

Some antibacterial drugs work by inhibiting nucleic acid synthesis ([\[link\]](#)). For example, **metronidazole** is a semisynthetic member of the nitroimidazole family that is also an antiprotozoan. It interferes with DNA replication in target cells. The drug **rifampin** is a semisynthetic member of the rifamycin family and functions by blocking RNA polymerase activity in bacteria. The RNA polymerase enzymes in bacteria are structurally different from those in eukaryotes, providing for selective toxicity against bacterial cells. It is used for the treatment of a variety of infections, but its primary use, often in a cocktail with other antibacterial drugs, is against mycobacteria that cause tuberculosis. Despite the selectivity of its mechanism, rifampin can induce liver enzymes to increase metabolism of other drugs being administered (antagonism), leading to hepatotoxicity (liver toxicity) and negatively influencing the bioavailability and therapeutic effect of the companion drugs.

One member of the quinolone family, a group of synthetic antimicrobials, is **nalidixic acid**. It was discovered in 1962 as a byproduct during the synthesis of chloroquine, an antimalarial drug. Nalidixic acid selectively inhibits the activity of bacterial DNA gyrase, blocking DNA replication. Chemical modifications to the original quinolone backbone have resulted in the production of **fluoroquinolones**, like ciprofloxacin and levofloxacin, which also inhibit the activity of DNA gyrase. Ciprofloxacin and levofloxacin are effective against a broad spectrum of gram-positive or gram-negative bacteria, and are among the most commonly prescribed antibiotics used to treat a wide range of infections, including urinary tract infections, respiratory infections, abdominal infections, and skin infections. However, despite their selective toxicity against DNA gyrase, side effects associated with different fluoroquinolones include phototoxicity, neurotoxicity, cardiotoxicity, glucose metabolism dysfunction, and increased risk for tendon rupture.

Drugs That Inhibit Bacterial Nucleic Acid Synthesis				
Mechanisms of Action	Drug Class	Specific Drugs	Spectrum of activity	Clinical Use
Inhibits bacterial RNA polymerase activity and blocks transcription, killing the cell	Rifamycin	Rifampin	Narrow spectrum with activity against gram-positive and limited numbers of gram-negative bacteria. Also active against <i>Mycobacterium tuberculosis</i> .	Combination therapy for treatment of tuberculosis
Inhibits the activity of DNA gyrase and blocks DNA replication, killing the cell	Fluoroquinolones	Ciprofloxacin, ofloxacin, moxifloxacin	Broad spectrum against gram-positive and gram-negative bacteria	Wide variety of skin and systemic infections

Note:

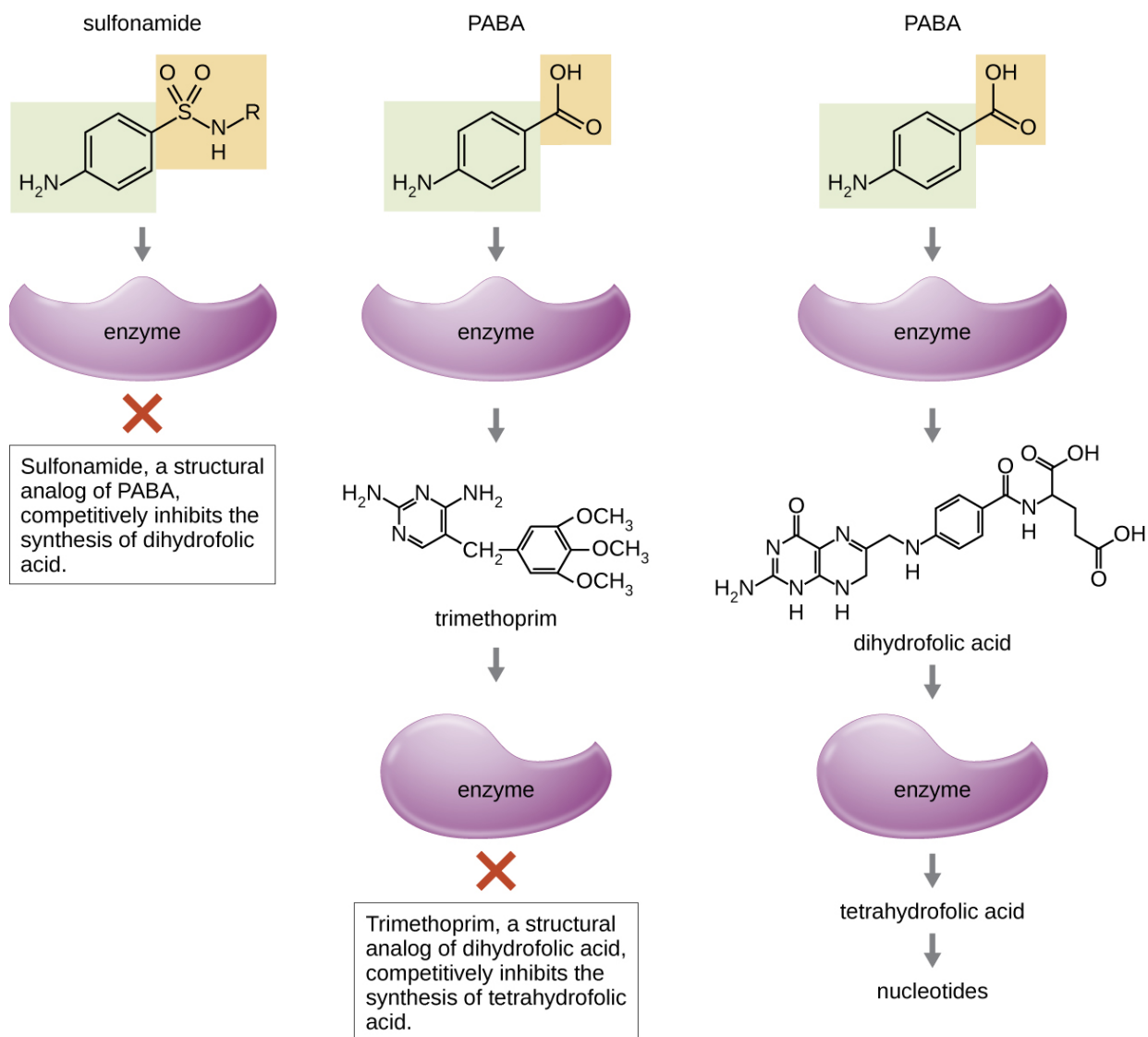
- Why do inhibitors of bacterial nucleic acid synthesis not target host cells?

Inhibitors of Metabolic Pathways

Some synthetic drugs control bacterial infections by functioning as **antimetabolites**, competitive inhibitors for bacterial metabolic enzymes ([\[link\]](#)). The **sulfonamides (sulfa drugs)** are the oldest synthetic antibacterial agents and are structural analogues of *para*-aminobenzoic acid (PABA), an early intermediate in folic acid synthesis ([\[link\]](#)). By inhibiting the enzyme involved in the production of dihydrofolic acid, sulfonamides block bacterial biosynthesis of folic acid and, subsequently, pyrimidines and purines required for nucleic acid synthesis. This mechanism of action provides bacteriostatic inhibition of growth against a wide spectrum of gram-positive and gram-negative pathogens. Because humans obtain folic acid from food instead of synthesizing it intracellularly, sulfonamides are selectively toxic for bacteria. However, allergic reactions to sulfa drugs are common. The sulfones are structurally similar to sulfonamides but are not commonly used today except for the treatment of Hansen's disease (leprosy).

Trimethoprim is a synthetic antimicrobial compound that serves as an antimetabolite within the same folic acid synthesis pathway as sulfonamides. However, **trimethoprim** is a structural analogue of dihydrofolic acid and inhibits a later step in the metabolic pathway ([\[link\]](#)). Trimethoprim is used in combination with the sulfa drug sulfamethoxazole to treat urinary tract infections, ear infections, and bronchitis. As discussed, the combination of trimethoprim and sulfamethoxazole is an example of antibacterial synergy. When used alone, each antimetabolite only decreases production of folic acid to a level where bacteriostatic inhibition of growth occurs. However, when used in combination, inhibition of both steps in the metabolic pathway decreases folic acid synthesis to a level that is lethal to the bacterial cell. Because of the importance of folic acid during fetal development, sulfa drugs and trimethoprim use should be carefully considered during early pregnancy.

The drug **isoniazid** is an antimetabolite with specific toxicity for mycobacteria and has long been used in combination with rifampin or streptomycin in the treatment of tuberculosis. It is administered as a prodrug, requiring activation through the action of an intracellular bacterial peroxidase enzyme, forming isoniazid-nicotinamide adenine dinucleotide (NAD) and isoniazid-nicotinamide adenine dinucleotide phosphate (NADP), ultimately preventing the synthesis of mycolic acid, which is essential for mycobacterial cell walls. Possible side effects of isoniazid use include hepatotoxicity, neurotoxicity, and hematologic toxicity (anemia).



Sulfonamides and trimethoprim are examples of antimetabolites that interfere in the bacterial synthesis of folic acid by blocking purine and pyrimidine biosynthesis, thus inhibiting bacterial growth.

Antimetabolite Drugs				
Metabolic Pathway Target	Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
Folic acid synthesis	Inhibits the enzyme involved in production of dihydrofolic acid	Sulfonamides	Sulfamethoxazole	Broad spectrum against gram-

Antimetabolite Drugs				
Metabolic Pathway Target	Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
		Sulfones	Dapsone	positive and gram-negative bacteria
	Inhibits the enzyme involved in the production of tetrahydrofolic acid	Not applicable	Trimethoprim	Broad spectrum against gram-positive and gram-negative bacteria
Mycolic acid synthesis	Interferes with the synthesis of mycolic acid	Not applicable	Isoniazid	Narrow spectrum against <i>Mycobacterium</i> spp., including <i>M. tuberculosis</i>

Note:

- How do sulfonamides and trimethoprim selectively target bacteria?

Inhibitor of ATP Synthase

Bedaquiline, representing the synthetic antibacterial class of compounds called the diarylquinolines, uses a novel mode of action that specifically inhibits mycobacterial growth. Although the specific mechanism has yet to be elucidated, this compound appears to interfere with the function of ATP synthases, perhaps by interfering with the use of the hydrogen ion gradient for ATP synthesis by oxidative phosphorylation, leading to reduced ATP production. Due to its side effects, including hepatotoxicity and potentially lethal heart arrhythmia, its use is reserved for serious, otherwise untreatable cases of tuberculosis.

Note:

To learn more about the general principles of antimicrobial therapy and bacterial modes of action, visit [Michigan State University's Antimicrobial Resistance Learning Site](#), particularly pages 6 through 9.

Note:

Part 2

Reading thorough Marisa's health history, the doctor noticed that during her hospitalization in Vietnam, she was catheterized and received the antimicrobial drugs ceftazidime and metronidazole. Upon learning this, the doctor ordered a CT scan of Marisa's abdomen to rule out appendicitis; the doctor also requested blood work to see if she

had an elevated white blood cell count, and ordered a urine analysis test and urine culture to look for the presence of white blood cells, red blood cells, and bacteria. Marisa's urine sample came back positive for the presence of bacteria, indicating a urinary tract infection (UTI). The doctor prescribed ciprofloxacin. In the meantime, her urine was cultured to grow the bacterium for further testing.

- What types of antimicrobials are typically prescribed for UTIs?
- Based upon the antimicrobial drugs she was given in Vietnam, which of the antimicrobials for treatment of a UTI would you predict to be ineffective?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Antibacterial compounds exhibit **selective toxicity**, largely due to differences between prokaryotic and eukaryotic cell structure.
- Cell wall synthesis inhibitors, including the **β -lactams**, the **glycopeptides**, and **bacitracin**, interfere with peptidoglycan synthesis, making bacterial cells more prone to osmotic lysis.
- There are a variety of broad-spectrum, bacterial protein synthesis inhibitors that selectively target the prokaryotic 70S ribosome, including those that bind to the 30S subunit (**aminoglycosides** and **tetracyclines**) and others that bind to the 50S subunit (**macrolides**, **lincosamides**, **chloramphenicol**, and **oxazolidinones**).
- **Polymyxins** are lipophilic polypeptide antibiotics that target the lipopolysaccharide component of gram-negative bacteria and ultimately disrupt the integrity of the outer and inner membranes of these bacteria.
- The nucleic acid synthesis inhibitors rifamycins and **fluoroquinolones** target bacterial RNA transcription and DNA replication, respectively.
- Some antibacterial drugs are **antimetabolites**, acting as competitive inhibitors for bacterial metabolic enzymes. **Sulfonamides** and **trimethoprim** are antimetabolites that interfere with bacterial folic acid synthesis. **Isoniazid** is an antimetabolite that interferes with mycolic acid synthesis in mycobacteria.

Multiple Choice

Exercise:

Problem:

Which of the following terms refers to the ability of an antimicrobial drug to harm the target microbe without harming the host?

- A. mode of action
- B. therapeutic level
- C. spectrum of activity
- D. selective toxicity

Solution:

D

Exercise:

Problem: Which of the following is not a type of β -lactam antimicrobial?

- A. penicillins
- B. glycopeptides
- C. cephalosporins

D. monobactams

Solution:

B

Exercise:

Problem: Which of the following does not bind to the 50S ribosomal subunit?

- A. tetracyclines
- B. lincosamides
- C. macrolides
- D. chloramphenicol

Solution:

A

Exercise:

Problem: Which of the following antimicrobials inhibits the activity of DNA gyrase?

- A. polymyxin B
- B. clindamycin
- C. nalidixic acid
- D. rifampin

Solution:

C

Fill in the Blank

Exercise:

Problem:

Selective toxicity antimicrobials are easier to develop against bacteria because they are _____ cells, whereas human cells are eukaryotic.

Solution:

prokaryotic

True/False

Exercise:

Problem: β -lactamases can degrade vancomycin.

Solution:

false

Short Answer

Exercise:

Problem:

If human cells and bacterial cells perform transcription, how are the rifamycins specific for bacterial infections?

Exercise:

Problem:

What bacterial structural target would make an antibacterial drug selective for gram-negative bacteria? Provide one example of an antimicrobial compound that targets this structure.

Critical Thinking

Exercise:

Problem:

In considering the cell structure of prokaryotes compared with that of eukaryotes, propose one possible reason for side effects in humans due to treatment of bacterial infections with protein synthesis inhibitors.

Mechanisms of Other Antimicrobial Drugs

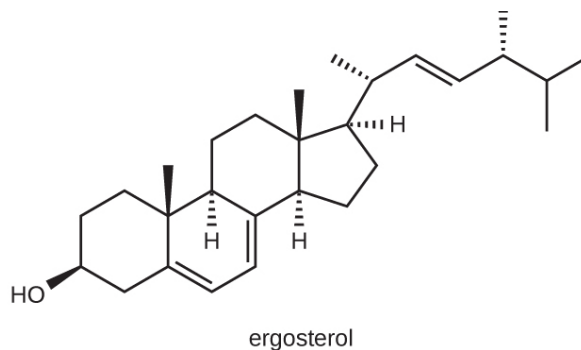
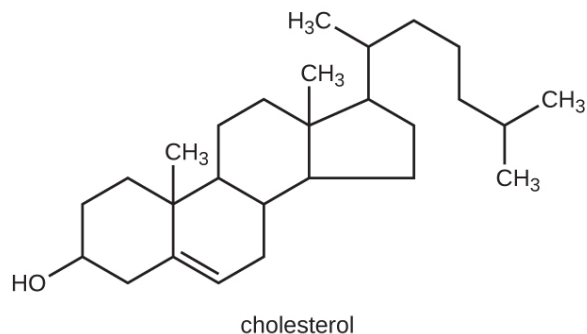
LEARNING OBJECTIVE

- Explain the differences between modes of action of drugs that target fungi, protozoa, helminths, and viruses

Because fungi, protozoa, and helminths are eukaryotic, their cells are very similar to human cells, making it more difficult to develop drugs with selective toxicity. Additionally, viruses replicate within human host cells, making it difficult to develop drugs that are selectively toxic to viruses or virus-infected cells. Despite these challenges, there are antimicrobial drugs that target fungi, protozoa, helminths, and viruses, and some even target more than one type of microbe. [\[link\]](#), [\[link\]](#), [\[link\]](#), and [\[link\]](#) provide examples for antimicrobial drugs in these various classes.

Antifungal Drugs

The most common mode of action for antifungal drugs is the disruption of the cell membrane. Antifungals take advantage of small differences between fungi and humans in the biochemical pathways that synthesize sterols. The sterols are important in maintaining proper membrane fluidity and, hence, proper function of the cell membrane. For most fungi, the predominant membrane sterol is ergosterol. Because human cell membranes use cholesterol, instead of ergosterol, antifungal drugs that target ergosterol synthesis are selectively toxic ([\[link\]](#)).



The predominant sterol found in human cells is cholesterol, whereas the predominant sterol found in fungi is ergosterol, making ergosterol a good target for antifungal drug development.

The **imidazoles** are synthetic fungicides that disrupt ergosterol biosynthesis; they are commonly used in medical applications and also in agriculture to keep seeds and harvested crops from molding. Examples include miconazole, ketoconazole, and clotrimazole, which are used to treat fungal skin infections such as ringworm, specifically tinea pedis (athlete's foot), tinea cruris (jock itch), and tinea corporis. These infections are commonly caused by dermatophytes of the genera *Trichophyton*, *Epidermophyton*, and *Microsporum*. Miconazole is also used predominantly for the treatment of vaginal yeast infections caused by the fungus *Candida*, and ketoconazole is used for the treatment of tinea versicolor and dandruff, which both can be caused by the fungus *Malassezia*.

The **triazole** drugs, including **fluconazole**, also inhibit ergosterol biosynthesis. However, they can be administered orally or intravenously for the treatment of several types of systemic yeast infections, including oral thrush and cryptococcal meningitis, both of which are prevalent in patients with AIDS. The triazoles also exhibit more selective toxicity, compared with the imidazoles, and are associated with fewer side effects.

The **allylamines**, a structurally different class of synthetic antifungal drugs, inhibit an earlier step in ergosterol biosynthesis. The most commonly used allylamine is **terbinafine** (marketed under the brand name Lamisil), which is used topically for the treatment of dermatophytic skin infections like athlete's foot, ringworm, and jock itch. Oral treatment with terbinafine is also used for the treatment of fingernail and toenail fungus, but it can be associated with the rare side effect of hepatotoxicity.

The **polyenes** are a class of antifungal agents naturally produced by certain actinomycete soil bacteria and are structurally related to macrolides. These large,

lipophilic molecules bind to ergosterol in fungal cytoplasmic membranes, thus creating pores. Common examples include nystatin and amphotericin B. Nystatin is typically used as a topical treatment for yeast infections of the skin, mouth, and vagina, but may also be used for intestinal fungal infections. The drug **amphotericin B** is used for systemic fungal infections like aspergillosis, cryptococcal meningitis, histoplasmosis, blastomycosis, and candidiasis. Amphotericin B was the only antifungal drug available for several decades, but its use is associated with some serious side effects, including nephrotoxicity (kidney toxicity).

Amphotericin B is often used in combination with flucytosine, a fluorinated pyrimidine analog that is converted by a fungal-specific enzyme into a toxic product that interferes with both DNA replication and protein synthesis in fungi. Flucytosine is also associated with hepatotoxicity (liver toxicity) and bone marrow depression.

Beyond targeting ergosterol in fungal cell membranes, there are a few antifungal drugs that target other fungal structures ([\[link\]](#)). The echinocandins, including caspofungin, are a group of naturally produced antifungal compounds that block the synthesis of $\beta(1 \rightarrow 3)$ glucan found in fungal cell walls but not found in human cells. This drug class has the nickname “penicillin for fungi.” Caspofungin is used for the treatment of aspergillosis as well as systemic yeast infections.

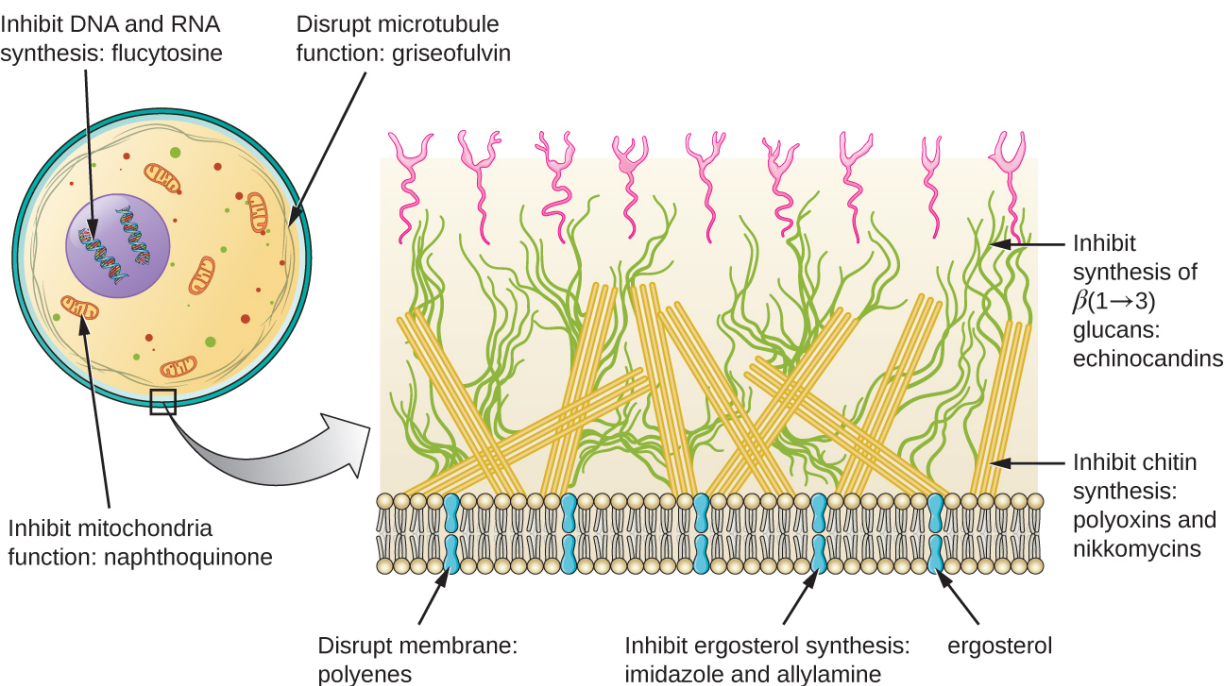
Although chitin is only a minor constituent of fungal cell walls, it is also absent in human cells, making it a selective target. The polyoxins and nikkomycins are naturally produced antifungals that target chitin synthesis. Polyoxins are used to control fungi for agricultural purposes, and nikkomycin Z is currently under development for use in humans to treat yeast infections and Valley fever (coccidioidomycosis), a fungal disease prevalent in the southwestern US.[\[footnote\]](#) Centers for Disease Control and Prevention. “Valley Fever: Awareness Is Key.” <http://www.cdc.gov/features/valleyfever/>. Accessed June 1, 2016.

The naturally produced antifungal griseofulvin is thought to specifically disrupt fungal cell division by interfering with microtubules involved in spindle formation during mitosis. It was one of the first antifungals, but its use is associated with hepatotoxicity. It is typically administered orally to treat various types of dermatophytic skin infections when other topical antifungal treatments are ineffective.

There are a few drugs that act as antimetabolites against fungal processes. For example, atovaquone, a representative of the naphthoquinone drug class, is a semisynthetic antimetabolite for fungal and protozoal versions of a mitochondrial cytochrome important in electron transport. Structurally, it is an analog of coenzyme Q, with which it competes for electron binding. It is particularly useful for the

treatment of *Pneumocystis* pneumonia caused by *Pneumocystis jirovecii*. The antibacterial sulfamethoxazole-trimethoprim combination also acts as an antimetabolite against *P. jirovecii*.

[\[link\]](#) shows the various therapeutic classes of antifungal drugs, categorized by mode of action, with examples of each.



Antifungal drugs target several different cell structures. (credit right: modification of work by “Maya and Rike”/Wikimedia Commons)

Common Antifungal Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses

Common Antifungal Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit ergosterol synthesis	Imidazoles	Miconazole, ketoconazole, clotrimazole	Fungal skin infections and vaginal yeast infections
	Triazoles	Fluconazole	Systemic yeast infections, oral thrush, and cryptococcal meningitis
	Allylamines	Terbinafine	Dermatophytic skin infections (athlete's foot, ring worm, jock itch), and infections of fingernails and toenails
Bind ergosterol in the cell membrane and create pores that disrupt the membrane	Polyenes	Nystatin	Used topically for yeast infections of skin, mouth, and vagina; also used for fungal infections of the intestine
		Amphotericin B	Variety systemic fungal infections
Inhibit cell wall synthesis	Echinocandins	Caspofungin	Aspergillosis and systemic yeast infections
	Not applicable	Nikkomycin Z	Coccidioidomycosis (Valley fever) and yeast infections

Common Antifungal Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit microtubules and cell division	Not applicable	Griseofulvin	Dermatophytic skin infections

Note:

- How is disruption of ergosterol biosynthesis an effective mode of action for antifungals?

Note:

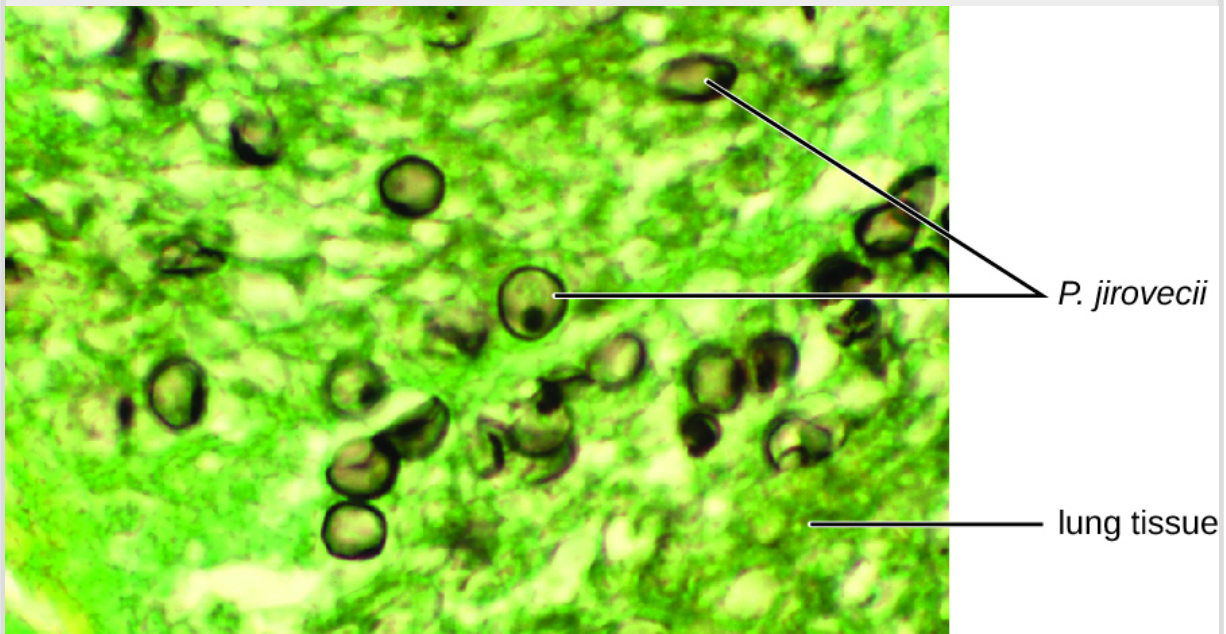
Treating a Fungal Infection of the Lungs

Jack, a 48-year-old engineer, is HIV positive but generally healthy thanks to antiretroviral therapy (ART). However, after a particularly intense week at work, he developed a fever and a dry cough. He assumed that he just had a cold or mild flu due to overexertion and didn't think much of it. However, after about a week, he began to experience fatigue, weight loss, and shortness of breath. He decided to visit his physician, who found that Jack had a low level of blood oxygenation. The physician ordered blood testing, a chest X-ray, and the collection of an induced sputum sample for analysis. His X-ray showed a fine cloudiness and several pneumatoceles (thin-walled pockets of air), which indicated *Pneumocystis pneumonia* (PCP), a type of pneumonia caused by the fungus *Pneumocystis jirovecii*. Jack's physician admitted him to the hospital and prescribed Bactrim, a combination of sulfamethoxazole and trimethoprim, to be administered intravenously.

P. jirovecii is a yeast-like fungus with a life cycle similar to that of protozoans. As such, it was classified as a protozoan until the 1980s. It lives only in the lung tissue of infected persons and is transmitted from person to person, with many people exposed as children. Typically, *P. jirovecii* only causes pneumonia in immunocompromised individuals. Healthy people may carry the fungus in their

lungs with no symptoms of disease. PCP is particularly problematic among HIV patients with compromised immune systems.

PCP is usually treated with oral or intravenous Bactrim, but atovaquone or pentamidine (another antiparasitic drug) are alternatives. If not treated, PCP can progress, leading to a collapsed lung and nearly 100% mortality. Even with antimicrobial drug therapy, PCP still is responsible for 10% of HIV-related deaths. The cytological examination, using direct immunofluorescence assay (DFA), of a smear from Jack's sputum sample confirmed the presence of *P. jirovecii* ([link](#)). Additionally, the results of Jack's blood tests revealed that his white blood cell count had dipped, making him more susceptible to the fungus. His physician reviewed his ART regimen and made adjustments. After a few days of hospitalization, Jack was released to continue his antimicrobial therapy at home. With the adjustments to his ART therapy, Jack's CD4 counts began to increase and he was able to go back to work.



Microscopic examination of an induced sputum sample or bronchoalveolar lavage sample typically reveals the organism, as shown here. (credit: modification of work by the Centers for Disease Control and Prevention)

Antiprotozoan Drugs

There are a few mechanisms by which antiprotozoan drugs target infectious protozoans ([link](#)). Some are antimetabolites, such as atovaquone, proguanil, and artemisinins. Atovaquone, in addition to being antifungal, blocks electron transport in protozoans and is used for the treatment of protozoan infections including malaria, babesiosis, and toxoplasmosis. Proguanil is another synthetic antimetabolite that is processed in parasitic cells into its active form, which inhibits protozoan folic acid synthesis. It is often used in combination with atovaquone, and the combination is marketed as Malarone for both malaria treatment and prevention.

Artemisinin, a plant-derived antifungal first discovered by Chinese scientists in the 1970s, is quite effective against malaria. Semisynthetic derivatives of **artemisinin** are more water soluble than the natural version, which makes them more bioavailable. Although the exact mechanism of action is unclear, artemisinins appear to act as prodrugs that are metabolized by target cells to produce reactive oxygen species (ROS) that damage target cells. Due to the rise in resistance to antimalarial drugs, artemisinins are also commonly used in combination with other antimalarial compounds in artemisinin-based combination therapy (ACT).

Several antimetabolites are used for the treatment of toxoplasmosis caused by the parasite *Toxoplasma gondii*. The synthetic sulfa drug sulfadiazine competitively inhibits an enzyme in folic acid production in parasites and can be used to treat malaria and toxoplasmosis. Pyrimethamine is a synthetic drug that inhibits a different enzyme in the folic acid production pathway and is often used in combination with sulfadoxine (another sulfa drug) for the treatment of malaria or in combination with sulfadiazine for the treatment of toxoplasmosis. Side effects of pyrimethamine include decreased bone marrow activity that may cause increased bruising and low red blood cell counts. When toxicity is a concern, spiramycin, a macrolide protein synthesis inhibitor, is typically administered for the treatment of toxoplasmosis.

Two classes of antiprotozoan drugs interfere with nucleic acid synthesis: nitroimidazoles and quinolines. Nitroimidazoles, including semisynthetic metronidazole, which was discussed previously as an antibacterial drug, and synthetic tinidazole, are useful in combating a wide variety of protozoan pathogens, such as *Giardia lamblia*, *Entamoeba histolytica*, and *Trichomonas vaginalis*. Upon introduction into these cells in low-oxygen environments, nitroimidazoles become activated and introduce DNA strand breakage, interfering with DNA replication in target cells. Unfortunately, metronidazole is associated with carcinogenesis (the development of cancer) in humans.

Another type of synthetic antiprotozoan drug that has long been thought to specifically interfere with DNA replication in certain pathogens is **pentamidine**. It has historically been used for the treatment of African sleeping sickness (caused by

the protozoan *Trypanosoma brucei*) and leishmaniasis (caused by protozoa of the genus *Leishmania*), but it is also an alternative treatment for the fungus *Pneumocystis*. Some studies indicate that it specifically binds to the DNA found within kinetoplasts (kDNA; long mitochondrion-like structures unique to trypanosomes), leading to the cleavage of kDNA. However, nuclear DNA of both the parasite and host remain unaffected. It also appears to bind to tRNA, inhibiting the addition of amino acids to tRNA, thus preventing protein synthesis. Possible side effects of pentamidine use include pancreatic dysfunction and liver damage.

The **quinolines** are a class of synthetic compounds related to quinine, which has a long history of use against malaria. Quinolines are thought to interfere with heme detoxification, which is necessary for the parasite’s effective breakdown of hemoglobin into amino acids inside red blood cells. The synthetic derivatives chloroquine, quinacrine (also called mepacrine), and mefloquine are commonly used as antimalarials, and chloroquine is also used to treat amebiasis typically caused by *Entamoeba histolytica*. Long-term prophylactic use of chloroquine or mefloquine may result in serious side effects, including hallucinations or cardiac issues. Patients with glucose-6-phosphate dehydrogenase deficiency experience severe anemia when treated with chloroquine.

Common Antiprotozoan Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit electron transport in mitochondria	Naphthoquinone	Atovaquone	Malaria, babesiosis, and toxoplasmosis
Inhibit folic acid synthesis	Not applicable	Proquanil	Combination therapy with atovaquone for malaria treatment and prevention

Common Antiprotozoan Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
	Sulfonamide	Sulfadiazine	Malaria and toxoplasmosis
	Not applicable	Pyrimethamine	Combination therapy with sulfadoxine (sulfa drug) for malaria
Produces damaging reactive oxygen species	Not applicable	Artemisinin	Combination therapy to treat malaria
Inhibit DNA synthesis	Nitroimidazoles	Metronidazole, tinidazole	Infections caused by <i>Giardia lamblia</i> , <i>Entamoeba histolytica</i> , and <i>Trichomonas vaginalis</i>
	Not applicable	Pentamidine	African sleeping sickness and leishmaniasis
Inhibit heme detoxification	Quinolines	Chloroquine	Malaria and infections with <i>E. histolytica</i>
		Mepacrine, mefloquine	Malaria

Note:

- List two modes of action for antiprotozoan drugs.

Antihelminthic Drugs

Because helminths are multicellular eukaryotes like humans, developing drugs with selective toxicity against them is extremely challenging. Despite this, several effective classes have been developed ([\[link\]](#)). Synthetic **benzimidazoles**, like **mebendazole** and **albendazole**, bind to helminthic β -tubulin, preventing microtubule formation. Microtubules in the intestinal cells of the worms seem to be particularly affected, leading to a reduction in glucose uptake. Besides their activity against a broad range of helminths, benzimidazoles are also active against many protozoans, fungi, and viruses, and their use for inhibiting mitosis and cell cycle progression in cancer cells is under study. [\[footnote\]](#) Possible side effects of their use include liver damage and bone marrow suppression.

B. Chu et al. "A Benzimidazole Derivative Exhibiting Antitumor Activity Blocks EGFR and HER2 Activity and Upregulates DR5 in Breast Cancer Cells." *Cell Death and Disease* 6 (2015):e1686

The avermectins are members of the macrolide family that were first discovered from a Japanese soil isolate, *Streptomyces avermectinius*. A more potent semisynthetic derivative of avermectin is **ivermectin**, which binds to glutamate-gated chloride channels specific to invertebrates including helminths, blocking neuronal transmission and causing starvation, paralysis, and death of the worms. Ivermectin is used to treat roundworm diseases, including onchocerciasis (also called river blindness, caused by the worm *Onchocerca volvulus*) and strongyloidiasis (caused by the worm *Strongyloides stercoralis* or *S. fuelleborni*). Ivermectin also can also treat parasitic insects like mites, lice, and bed bugs, and is nontoxic to humans.

Niclosamide is a synthetic drug that has been used for over 50 years to treat tapeworm infections. Although its mode of action is not entirely clear, niclosamide appears to inhibit ATP formation under anaerobic conditions and inhibit oxidative phosphorylation in the mitochondria of its target pathogens. Niclosamide is not absorbed from the gastrointestinal tract, thus it can achieve high localized intestinal concentrations in patients. Recently, it has been shown to also have antibacterial, antiviral, and antitumor activities. [\[footnote\]](#)[\[footnote\]](#)[\[footnote\]](#)

J.-X. Pan et al. "Niclosamide, An Old Antihelminthic Agent, Demonstrates Antitumor Activity by Blocking Multiple Signaling Pathways of Cancer Stem Cells."

Chinese Journal of Cancer 31 no. 4 (2012):178–184.

F. Imperi et al. “New Life for an Old Drug: The Anthelmintic Drug Niclosamide Inhibits *Pseudomonas aeruginosa* Quorum Sensing.” *Antimicrobial Agents and Chemotherapy* 57 no. 2 (2013):996-1005.

A. Jurgeit et al. “Niclosamide Is a Proton Carrier and Targets Acidic Endosomes with Broad Antiviral Effects.” *PLoS Pathogens* 8 no. 10 (2012):e1002976.

Another synthetic antihelminthic drug is **praziquantel**, which used for the treatment of parasitic tapeworms and liver flukes, and is particularly useful for the treatment of schistosomiasis (caused by blood flukes from three genera of *Schistosoma*). Its mode of action remains unclear, but it appears to cause the influx of calcium into the worm, resulting in intense spasm and paralysis of the worm. It is often used as a preferred alternative to niclosamide in the treatment of tapeworms when gastrointestinal discomfort limits niclosamide use.

The thioxanthenones, another class of synthetic drugs structurally related to quinine, exhibit antischistosomal activity by inhibiting RNA synthesis. The thioxanthenone lucanthone and its metabolite hycanthone were the first used clinically, but serious neurological, gastrointestinal, cardiovascular, and hepatic side effects led to their discontinuation. Oxamniquine, a less toxic derivative of hycanthone, is only effective against *S. mansoni*, one of the three species known to cause schistosomiasis in humans. Praziquantel was developed to target the other two schistosome species, but concerns about increasing resistance have renewed interest in developing additional derivatives of oxamniquine to target all three clinically important schistosome species.

Common Antihelminthic Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit microtubule formation, reducing glucose uptake	Benzimidazoles	Mebendazole, albendazole	Variety of helminth infections

Common Antihelminthic Drugs			
Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Block neuronal transmission, causing paralysis and starvation	Avermectins	Ivermectin	Roundworm diseases, including river blindness and strongyloidiasis, and treatment of parasitic insects
Inhibit ATP production	Not applicable	Niclosamide	Intestinal tapeworm infections
Induce calcium influx	Not applicable	Praziquantel	Schistosomiasis (blood flukes)
Inhibit RNA synthesis	Thioxanthenones	Lucanthone, hycanthone, oxamniquine	Schistosomiasis (blood flukes)

Note:

- Why are antihelminthic drugs difficult to develop?

Antiviral Drugs

Unlike the complex structure of fungi, protozoa, and helminths, viral structure is simple, consisting of nucleic acid, a protein coat, viral enzymes, and, sometimes, a lipid envelope. Furthermore, viruses are obligate intracellular pathogens that use the host's cellular machinery to replicate. These characteristics make it difficult to develop drugs with selective toxicity against viruses.

Many antiviral drugs are nucleoside analogs and function by inhibiting nucleic acid biosynthesis. For example, **acyclovir** (marketed as Zovirax) is a synthetic analog of the nucleoside guanosine ([link](#)). It is activated by the herpes simplex viral enzyme thymidine kinase and, when added to a growing DNA strand during replication, causes chain termination. Its specificity for virus-infected cells comes from both the need for a viral enzyme to activate it and the increased affinity of the activated form for viral DNA polymerase compared to host cell DNA polymerase. Acyclovir and its derivatives are frequently used for the treatment of herpes virus infections, including genital herpes, chickenpox, shingles, Epstein-Barr virus infections, and cytomegalovirus infections. Acyclovir can be administered either topically or systemically, depending on the infection. One possible side effect of its use includes nephrotoxicity. The drug adenine-arabinoside, marketed as vidarabine, is a synthetic analog to deoxyadenosine that has a mechanism of action similar to that of acyclovir. It is also effective for the treatment of various human herpes viruses. However, because of possible side effects involving low white blood cell counts and neurotoxicity, treatment with acyclovir is now preferred.

Ribavirin, another synthetic guanosine analog, works by a mechanism of action that is not entirely clear. It appears to interfere with both DNA and RNA synthesis, perhaps by reducing intracellular pools of guanosine triphosphate (GTP). Ribavirin also appears to inhibit the RNA polymerase of hepatitis C virus. It is primarily used for the treatment of the RNA viruses like hepatitis C (in combination therapy with interferon) and respiratory syncytial virus. Possible side effects of ribavirin use include anemia and developmental effects on unborn children in pregnant patients. In recent years, another nucleotide analog, sofosbuvir (Solvaldi), has also been developed for the treatment of hepatitis C. Sofosbuvir is a uridine analog that interferes with viral polymerase activity. It is commonly coadministered with ribavirin, with and without interferon.

Inhibition of nucleic acid synthesis is not the only target of synthetic antivirals. Although the mode of action of **amantadine** and its relative **rimantadine** are not entirely clear, these drugs appear to bind to a transmembrane protein that is involved in the escape of the influenza virus from endosomes. Blocking escape of the virus also prevents viral RNA release into host cells and subsequent viral replication. Increasing resistance has limited the use of amantadine and rimantadine in the treatment of influenza A. Use of amantadine can result in neurological side effects, but the side effects of rimantadine seem less severe. Interestingly, because of their effects on brain chemicals such as dopamine and NMDA (N-methyl D-aspartate), amantadine and rimantadine are also used for the treatment of Parkinson's disease.

Neuraminidase inhibitors, including oseltamivir (Tamiflu), zanamivir (Relenza), and peramivir (Rapivab), specifically target influenza viruses by blocking the activity of

influenza virus neuraminidase, preventing the release of the virus from infected cells. These three antivirals can decrease flu symptoms and shorten the duration of illness, but they differ in their modes of administration: oseltamivir is administered orally, zanamivir is inhaled, and peramivir is administered intravenously. Resistance to these neuraminidase inhibitors still seems to be minimal.

Pleconaril is a synthetic antiviral under development that showed promise for the treatment of picornaviruses. Use of **pleconaril** for the treatment of the common cold caused by rhinoviruses was not approved by the FDA in 2002 because of lack of proven effectiveness, lack of stability, and association with irregular menstruation. Its further development for this purpose was halted in 2007. However, pleconaril is still being investigated for use in the treatment of life-threatening complications of enteroviruses, such as meningitis and sepsis. It is also being investigated for use in the global eradication of a specific enterovirus, polio.[\[footnote\]](#) Pleconaril seems to work by binding to the viral capsid and preventing the uncoating of viral particles inside host cells during viral infection.

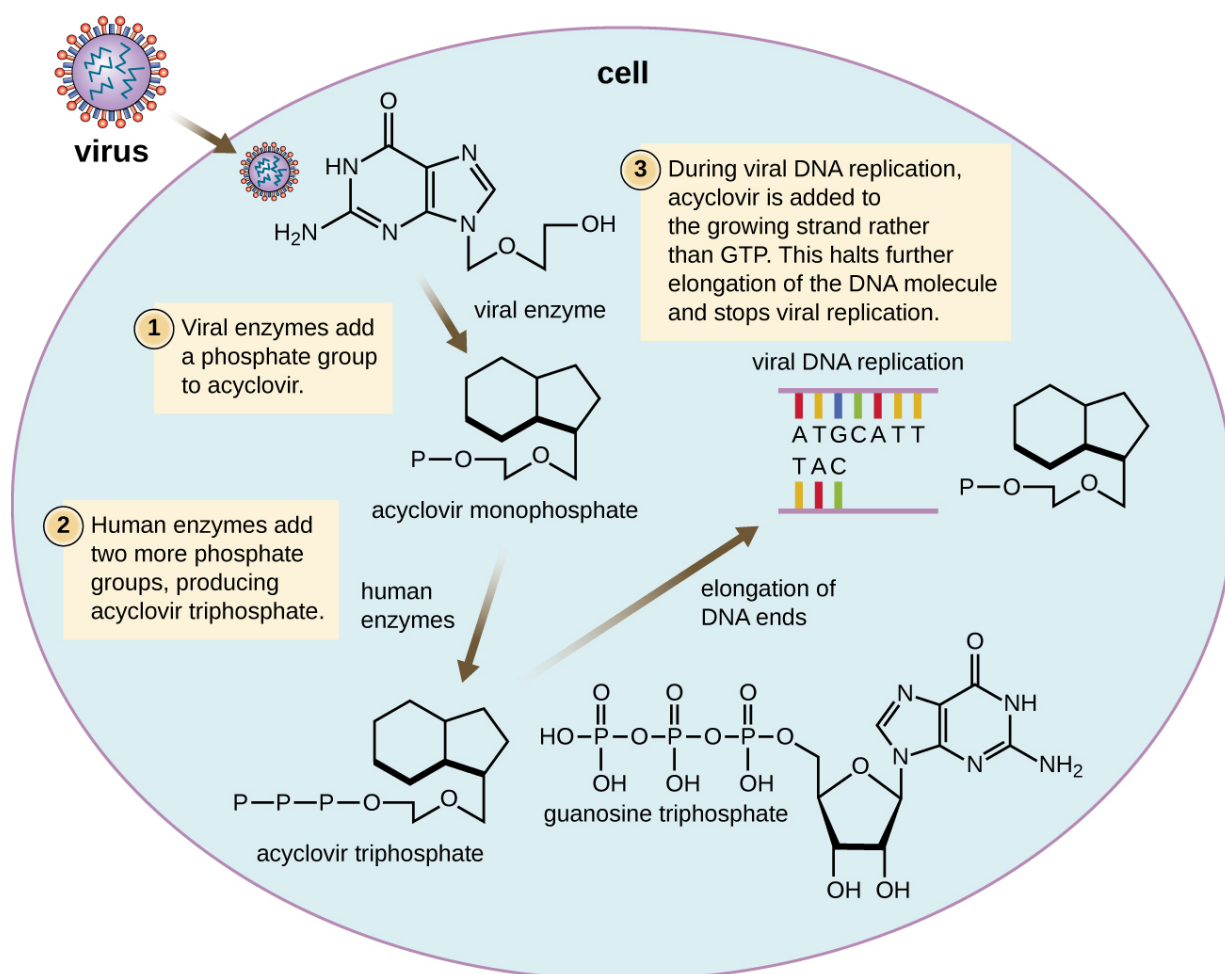
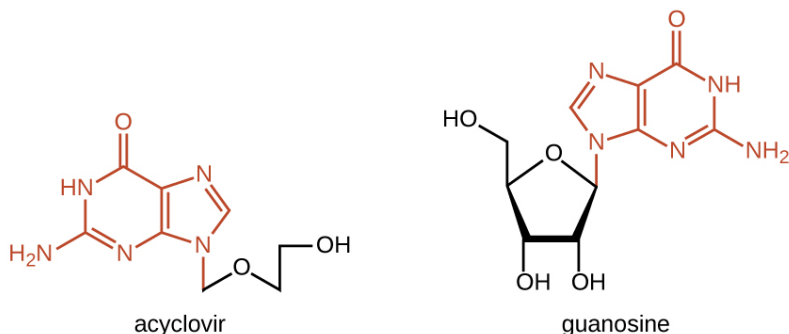
M.J. Abzug. “The Enteroviruses: Problems in Need of Treatments.” *Journal of Infection* 68 no. S1 (2014):108–14.

Viruses with complex life cycles, such as HIV, can be more difficult to treat. First, HIV targets CD4-positive white blood cells, which are necessary for a normal immune response to infection. Second, HIV is a retrovirus, meaning that it converts its RNA genome into a DNA copy that integrates into the host cell’s genome, thus hiding within host cell DNA. Third, the HIV reverse transcriptase lacks proofreading activity and introduces mutations that allow for rapid development of antiviral drug resistance. To help prevent the emergence of resistance, a combination of specific synthetic antiviral drugs is typically used in ART for HIV ([\[link\]](#)).

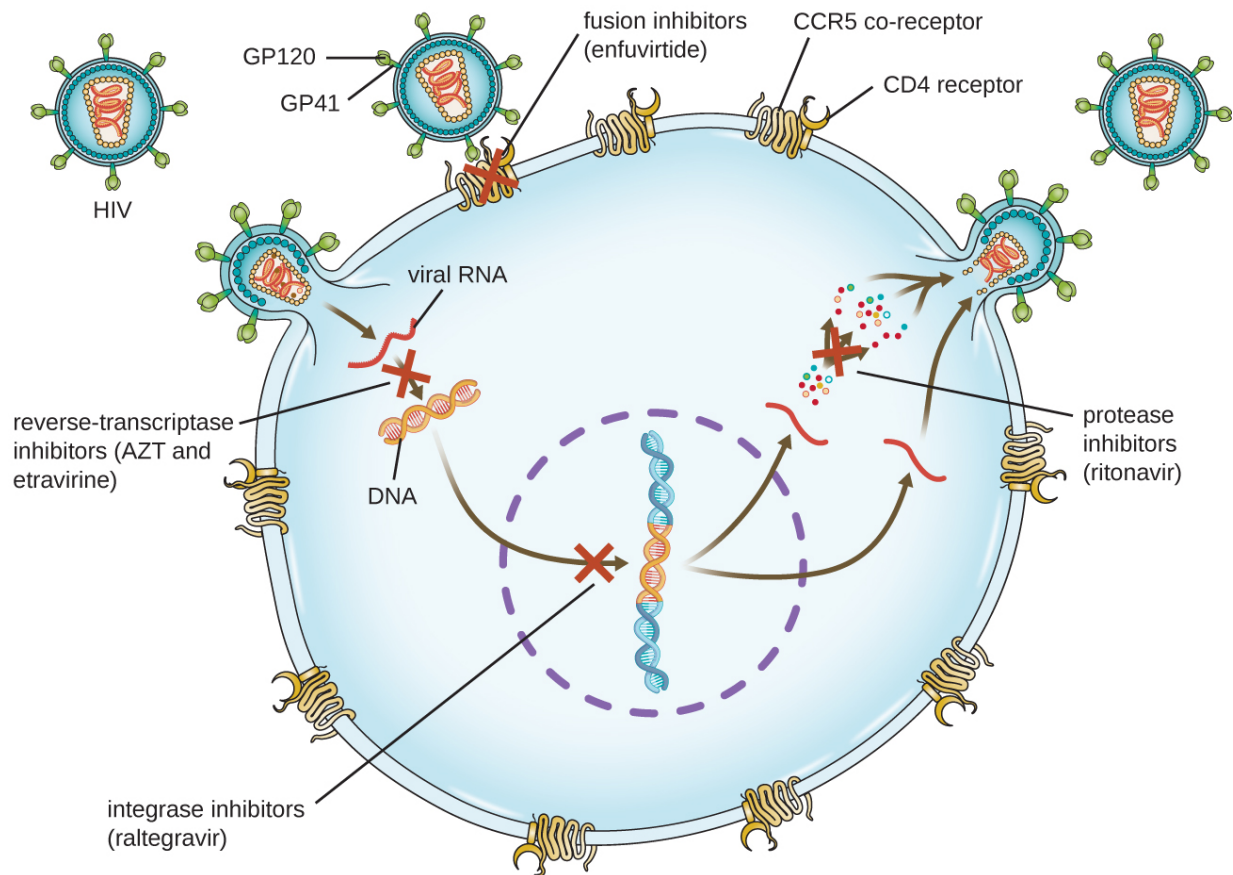
The **reverse transcriptase inhibitors** block the early step of converting viral RNA genome into DNA, and can include competitive nucleoside analog inhibitors (e.g., azidothymidine/zidovudine, or AZT) and non-nucleoside noncompetitive inhibitors (e.g., etravirine) that bind reverse transcriptase and cause an inactivating conformational change. Drugs called **protease inhibitors** (e.g., ritonavir) block the processing of viral proteins and prevent viral maturation. Protease inhibitors are also being developed for the treatment of other viral types.[\[footnote\]](#) For example, simeprevir (Olysio) has been approved for the treatment of hepatitis C and is administered with ribavirin and interferon in combination therapy. The **integrase inhibitors** (e.g., raltegravir), block the activity of the HIV integrase responsible for the recombination of a DNA copy of the viral genome into the host cell chromosome. Additional drug classes for HIV treatment include the CCR5 antagonists and the **fusion inhibitors** (e.g., enfuvirtide), which prevent the binding of HIV to the host cell coreceptor (chemokine receptor type 5 [CCR5]) and the merging of the viral

envelope with the host cell membrane, respectively. [\[link\]](#) shows the various therapeutic classes of antiviral drugs, categorized by mode of action, with examples of each.

B.L. Pearlman. "Protease Inhibitors for the Treatment of Chronic Hepatitis C Genotype-1 Infection: The New Standard of Care." *Lancet Infectious Diseases* 12 no. 9 (2012):717–728.



Acyclovir is a structural analog of guanosine. It is specifically activated by the viral enzyme thymidine kinase and then preferentially binds to viral DNA polymerase, leading to chain termination during DNA replication.



Antiretroviral therapy (ART) is typically used for the treatment of HIV. The targets of drug classes currently in use are shown here. (credit: modification of work by Thomas Spletstoesser)

Common Antiviral Drugs		
Mechanism of Action	Drug	Clinical Uses
Nucleoside analog inhibition of nucleic acid synthesis	Acyclovir	Herpes virus infections
	Azidothymidine/zidovudine (AZT)	HIV infections
	Ribavirin	Hepatitis C virus and respiratory syncytial virus infections
	Vidarabine	Herpes virus infections
	Sofosbuvir	Hepatitis C virus infections
Non-nucleoside noncompetitive inhibition	Etravirine	HIV infections
Inhibit escape of virus from endosomes	Amantadine, rimantadine	Infections with influenza virus
Inhibit neuraminidase	Oseltamivir, zanamivir, peramivir	Infections with influenza virus
Inhibit viral uncoating	Pleconaril	Serious enterovirus infections
Inhibition of protease	Ritonavir	HIV infections
	Simeprevir	Hepatitis C virus infections

Common Antiviral Drugs		
Mechanism of Action	Drug	Clinical Uses
Inhibition of integrase	Raltegravir	HIV infections
Inhibition of membrane fusion	Enfuvirtide	HIV infections

Note:

- Why is HIV difficult to treat with antivirals?

Note:

To learn more about the various classes of antiretroviral drugs used in the ART of HIV infection, explore each of the drugs in the HIV drug classes provided by US Department of Health and Human Services at [this](#) website.

Key Concepts and Summary

- Because fungi, protozoans, and helminths are eukaryotic organisms like human cells, it is more challenging to develop antimicrobial drugs that specifically target them. Similarly, it is hard to target viruses because human viruses replicate inside of human cells.
- **Antifungal drugs** interfere with ergosterol synthesis, bind to ergosterol to disrupt fungal cell membrane integrity, or target cell wall-specific components or other cellular proteins.
- **Antiprotozoan drugs** increase cellular levels of reactive oxygen species, interfere with protozoal DNA replication (nuclear versus kDNA, respectively), and disrupt heme detoxification.
- **Anthelmintic drugs** disrupt helminthic and protozoan microtubule formation; block neuronal transmissions; inhibit anaerobic ATP formation and/or oxidative phosphorylation; induce a calcium influx in tapeworms, leading to spasms and paralysis; and interfere with RNA synthesis in schistosomes.

- **Antiviral drugs** inhibit viral entry, inhibit viral uncoating, inhibit nucleic acid biosynthesis, prevent viral escape from endosomes in host cells, and prevent viral release from infected cells.
- Because it can easily mutate to become drug resistant, HIV is typically treated with a combination of several **antiretroviral drugs**, which may include **reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors**, and drugs that interfere with viral binding and fusion to initiate infection.

Multiple Choice

Exercise:

Problem:

Which of the following is not an appropriate target for antifungal drugs?

- A. ergosterol
- B. chitin
- C. cholesterol
- D. $\beta(1 \rightarrow 3)$ glucan

Solution:

C

Exercise:

Problem:

Which of the following drug classes specifically inhibits neuronal transmission in helminths?

- A. quinolines
- B. avermectins
- C. amantadines
- D. imidazoles

Solution:

B

Exercise:

Problem:

Which of the following is a nucleoside analog commonly used as a reverse transcriptase inhibitor in the treatment of HIV?

- A. acyclovir
- B. ribavirin
- C. adenine-arabinoside
- D. azidothymidine

Solution:

D

Exercise:**Problem:**

Which of the following is an antimalarial drug that is thought to increase ROS levels in target cells?

- A. artemisinin
- B. amphotericin b
- C. praziquantel
- D. pleconaril

Solution:

A

Fill in the Blank**Exercise:****Problem:**

Antiviral drugs, like Tamiflu and Relenza, that are effective against the influenza virus by preventing viral escape from host cells are called _____.

Solution:

neuraminidase inhibitors

True/False

Exercise:

Problem:

Echinocandins, known as “penicillin for fungi,” target $\beta(1 \rightarrow 3)$ glucan in fungal cell walls.

Solution:

true

Short Answer

Exercise:

Problem:

How does the biology of HIV necessitate the need to treat HIV infections with multiple drugs?

Exercise:

Problem:

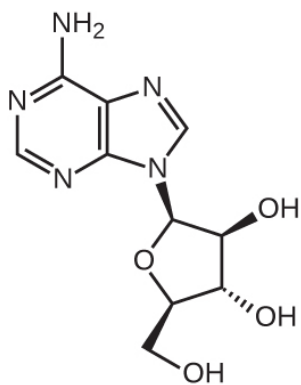
Niclosamide is insoluble and thus is not readily absorbed from the stomach into the bloodstream. How does the insolubility of niclosamide aid its effectiveness as a treatment for tapeworm infection?

Critical Thinking

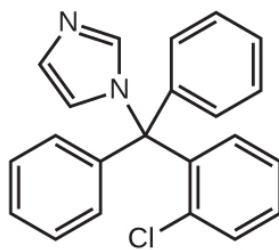
Exercise:

Problem:

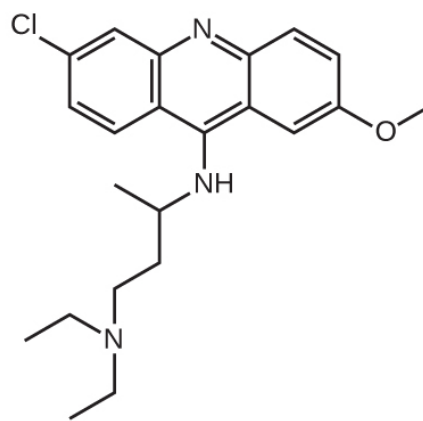
Which of the following molecules is an example of a nucleoside analog?



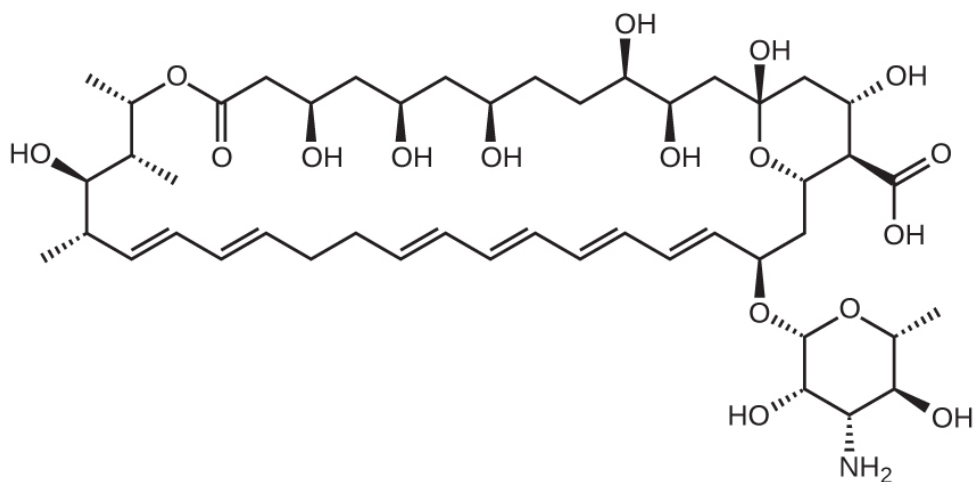
A



B



C



D

Exercise:

Problem:

Why can't drugs used to treat influenza, like amantadines and neuraminidase inhibitors, be used to treat a wider variety of viral infections?

Drug Resistance

LEARNING OBJECTIVES

- Explain the concept of drug resistance
- Describe how microorganisms develop or acquire drug resistance
- Describe the different mechanisms of antimicrobial drug resistance

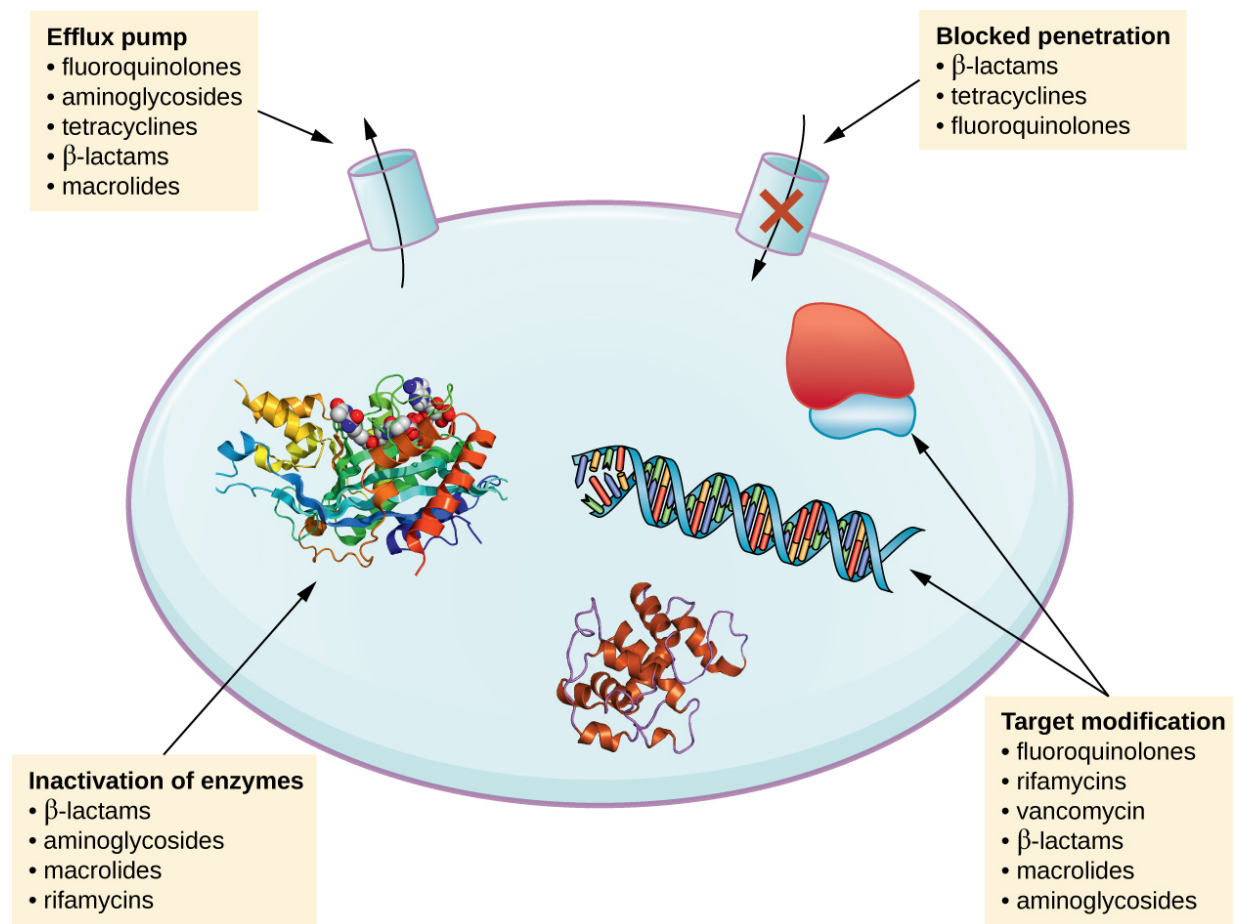
Antimicrobial resistance is not a new phenomenon. In nature, microbes are constantly evolving in order to overcome the antimicrobial compounds produced by other microorganisms. Human development of antimicrobial drugs and their widespread clinical use has simply provided another selective pressure that promotes further evolution. Several important factors can accelerate the evolution of **drug resistance**. These include the overuse and misuse of antimicrobials, inappropriate use of antimicrobials, subtherapeutic dosing, and patient noncompliance with the recommended course of treatment.

Exposure of a pathogen to an antimicrobial compound can select for chromosomal mutations conferring resistance, which can be transferred vertically to subsequent microbial generations and eventually become predominant in a microbial population that is repeatedly exposed to the antimicrobial. Alternatively, many genes responsible for drug resistance are found on plasmids or in transposons that can be transferred easily between microbes through horizontal gene transfer (see [How Asexual Prokaryotes Achieve Genetic Diversity](#)). Transposons also have the ability to move

resistance genes between plasmids and chromosomes to further promote the spread of resistance.

Mechanisms for Drug Resistance

There are several common mechanisms for drug resistance, which are summarized in [\[link\]](#). These mechanisms include enzymatic modification of the drug, modification of the antimicrobial target, and prevention of drug penetration or accumulation.



There are multiple strategies that microbes use to develop resistance to antimicrobial drugs. (Not shown: target overproduction, target mimicry, and enzymatic bypass). (credit: modification of work by Gerard D Wright)

Drug Modification or Inactivation

Resistance genes may code for enzymes that chemically modify an antimicrobial, thereby inactivating it, or destroy an antimicrobial through hydrolysis. Resistance to many types of antimicrobials occurs through this mechanism. For example, aminoglycoside resistance can occur through enzymatic transfer of chemical groups to the drug molecule, impairing the binding of the drug to its bacterial target. For β -lactams, bacterial resistance can involve the enzymatic hydrolysis of the β -lactam bond within the β -lactam ring of the drug molecule. Once the β -lactam bond is broken, the drug loses its antibacterial activity. This mechanism of resistance is mediated by β -lactamases, which are the most common mechanism of β -lactam resistance. Inactivation of rifampin commonly occurs through glycosylation, phosphorylation, or adenosine diphosphate (ADP) ribosylation, and resistance to macrolides and lincosamides can also occur due to enzymatic inactivation of the drug or modification.

Prevention of Cellular Uptake or Efflux

Microbes may develop resistance mechanisms that involve inhibiting the accumulation of an antimicrobial drug, which then prevents the drug from reaching its cellular target. This strategy is common among gram-negative pathogens and can involve changes in outer membrane lipid composition, porin channel selectivity, and/or porin channel concentrations. For example, a common mechanism of carbapenem resistance among *Pseudomonas aeruginosa* is to decrease the amount of its OprD porin, which is the primary portal of entry for carbapenems through the outer membrane of this pathogen. Additionally, many gram-positive and gram-negative pathogenic bacteria produce efflux pumps that actively transport an antimicrobial drug out of the cell and prevent the accumulation of drug to a level that would be antibacterial. For example, resistance to β -lactams, tetracyclines, and

fluoroquinolones commonly occurs through active efflux out of the cell, and it is rather common for a single efflux pump to have the ability to translocate multiple types of antimicrobials.

Target Modification

Because antimicrobial drugs have very specific targets, structural changes to those targets can prevent drug binding, rendering the drug ineffective. Through spontaneous mutations in the genes encoding antibacterial drug targets, bacteria have an evolutionary advantage that allows them to develop resistance to drugs. This mechanism of resistance development is quite common. Genetic changes impacting the active site of penicillin-binding proteins (PBPs) can inhibit the binding of β -lactam drugs and provide resistance to multiple drugs within this class. This mechanism is very common among strains of *Streptococcus pneumoniae*, which alter their own PBPs through genetic mechanisms. In contrast, strains of *Staphylococcus aureus* develop resistance to methicillin (MRSA) through the acquisition of a new low-affinity PBP, rather than structurally alter their existing PBPs. Not only does this new low-affinity PBP provide resistance to methicillin but it provides resistance to virtually all β -lactam drugs, with the exception of the newer fifth-generation cephalosporins designed specifically to kill MRSA. Other examples of this resistance strategy include alterations in

- ribosome subunits, providing resistance to macrolides, tetracyclines, and aminoglycosides;
- lipopolysaccharide (LPS) structure, providing resistance to polymyxins;
- RNA polymerase, providing resistance to rifampin;
- DNA gyrase, providing resistance to fluoroquinolones;
- metabolic enzymes, providing resistance to sulfa drugs, sulfones, and trimethoprim; and
- peptidoglycan subunit peptide chains, providing resistance to glycopeptides.

Target Overproduction or Enzymatic Bypass

When an antimicrobial drug functions as an antimetabolite, targeting a specific enzyme to inhibit its activity, there are additional ways that microbial resistance may occur. First, the microbe may overproduce the target enzyme such that there is a sufficient amount of antimicrobial-free enzyme to carry out the proper enzymatic reaction. Second, the bacterial cell may develop a bypass that circumvents the need for the functional target enzyme. Both of these strategies have been found as mechanisms of sulfonamide resistance. Vancomycin resistance among *S. aureus* has been shown to involve the decreased cross-linkage of peptide chains in the bacterial cell wall, which provides an increase in targets for vancomycin to bind to in the outer cell wall. Increased binding of vancomycin in the outer cell wall provides a blockage that prevents free drug molecules from penetrating to where they can block new cell wall synthesis.

Target Mimicry

A recently discovered mechanism of resistance called target mimicry involves the production of proteins that bind and sequester drugs, preventing the drugs from binding to their target. For example, *Mycobacterium tuberculosis* produces a protein with regular pentapeptide repeats that appears to mimic the structure of DNA. This protein binds fluoroquinolones, sequestering them and keeping them from binding to DNA, providing *M. tuberculosis* resistance to fluoroquinolones. Proteins that mimic the A-site of the bacterial ribosome have been found to contribute to aminoglycoside resistance as well. [\[footnote\]](#)

D.H. Fong, A.M. Berghuis. “Substrate Promiscuity of an Aminoglycoside Antibiotic Resistance Enzyme Via Target Mimicry.” *EMBO Journal* 21 no. 10 (2002):2323–2331.

Note:

- List several mechanisms for drug resistance.

Multidrug-Resistant Microbes and Cross Resistance

From a clinical perspective, our greatest concerns are **multidrug-resistant microbes (MDRs)** and cross resistance. MDRs are colloquially known as “superbugs” and carry one or more resistance mechanism(s), making them resistant to multiple antimicrobials. In **cross-resistance**, a single resistance mechanism confers resistance to multiple antimicrobial drugs. For example, having an efflux pump that can export multiple antimicrobial drugs is a common way for microbes to be resistant to multiple drugs by using a single resistance mechanism. In recent years, several clinically important superbugs have emerged, and the CDC reports that superbugs are responsible for more than 2 million infections in the US annually, resulting in at least 23,000 fatalities. [\[footnote\]](#) Several of the superbugs discussed in the following sections have been dubbed the ESKAPE pathogens. This acronym refers to the names of the pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) but it is also fitting in that these pathogens are able to “escape” many conventional forms of antimicrobial therapy. As such, infections by ESKAPE pathogens can be difficult to treat and they cause a large number of nosocomial infections. Centers for Disease Control and Prevention. “Antibiotic/Antimicrobial Resistance.” <http://www.cdc.gov/drugresistance/index.html>. Accessed June 2, 2016.

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Methicillin, a semisynthetic penicillin, was designed to resist inactivation by β -lactamases. Unfortunately, soon after the introduction of methicillin to clinical practice, methicillin-resistant strains of *S. aureus* appeared and started to spread. The mechanism of resistance, acquisition of a new low-affinity PBP, provided *S. aureus* with resistance to all available β -lactams. Strains of **methicillin-resistant *S. aureus* (MRSA)** are widespread opportunistic pathogens and a particular concern for skin and other wound infections, but may also cause pneumonia and septicemia. Although

originally a problem in health-care settings (hospital-acquired MRSA [HA-MRSA]), MRSA infections are now also acquired through contact with contaminated members of the general public, called community-associated MRSA (CA-MRSA). Approximately one-third of the population carries *S. aureus* as a member of their normal nasal microbiota without illness, and about 6% of these strains are methicillin resistant. [\[footnote\]](#)[\[footnote\]](#)

A.S. Kalokhe et al. “Multidrug-Resistant Tuberculosis Drug Susceptibility and Molecular Diagnostic Testing: A Review of the Literature. *American Journal of the Medical Sciences* 345 no. 2 (2013):143–148.

Centers for Disease Control and Prevention. “Methicillin-Resistant *Staphylococcus aureus* (MRSA): General Information About MRSA in the Community.” <http://www.cdc.gov/mrsa/community/index.html>. Accessed June 2, 2016

Note:

Clavulanic Acid: Penicillin’s Little Helper

With the introduction of penicillin in the early 1940s, and its subsequent mass production, society began to think of antibiotics as miracle cures for a wide range of infectious diseases. Unfortunately, as early as 1945, penicillin resistance was first documented and started to spread. Greater than 90% of current *S. aureus* clinical isolates are resistant to penicillin.

[\[footnote\]](#)

F.D. Lowy. “Antimicrobial Resistance: The Example of *Staphylococcus aureus*.” *Journal of Clinical Investigation* 111 no. 9 (2003):1265–1273.

Although developing new antimicrobial drugs is one solution to this problem, scientists have explored new approaches, including the development of compounds that inactivate resistance mechanisms. The development of clavulanic acid represents an early example of this strategy. Clavulanic acid is a molecule produced by the bacterium *Streptococcus clavuligerus*. It contains a β -lactam ring, making it structurally similar to penicillin and other β -lactams, but shows no clinical effectiveness when administered on its own. Instead, clavulanic acid binds irreversibly within the active site of β -lactamases and prevents them from inactivating a coadministered penicillin.

Clavulanic acid was first developed in the 1970s and was mass marketed in combination with amoxicillin beginning in the 1980s under the brand name Augmentin. As is typically the case, resistance to the amoxicillin-clavulanic acid combination soon appeared. Resistance most commonly results from bacteria increasing production of their β -lactamase and overwhelming the inhibitory effects of clavulanic acid, mutating their β -lactamase so it is no longer inhibited by clavulanic acid, or from acquiring a new β -lactamase that is not inhibited by clavulanic acid. Despite increasing resistance concerns, clavulanic acid and related β -lactamase inhibitors (sulbactam and tazobactam) represent an important new strategy: the development of compounds that directly inhibit antimicrobial resistance-conferring enzymes.

Vancomycin-Resistant Enterococci and *Staphylococcus aureus*

Vancomycin is only effective against gram-positive organisms, and it is used to treat wound infections, septic infections, endocarditis, and meningitis that are caused by pathogens resistant to other antibiotics. It is considered one of the last lines of defense against such resistant infections, including MRSA. With the rise of antibiotic resistance in the 1970s and 1980s, vancomycin use increased, and it is not surprising that we saw the emergence and spread of **vancomycin-resistant enterococci (VRE)**, **vancomycin-resistant *S. aureus* (VRSA)**, and **vancomycin-intermediate *S. aureus* (VISA)**. The mechanism of vancomycin resistance among enterococci is target modification involving a structural change to the peptide component of the peptidoglycan subunits, preventing vancomycin from binding. These strains are typically spread among patients in clinical settings by contact with health-care workers and contaminated surfaces and medical equipment.

VISA and VRSA strains differ from each other in the mechanism of resistance and the degree of resistance each mechanism confers. VISA strains exhibit intermediate resistance, with a minimum inhibitory concentration (MIC) of 4–8 $\mu\text{g/mL}$, and the mechanism involves an increase in vancomycin targets. VISA strains decrease the crosslinking of

peptide chains in the cell wall, providing an increase in vancomycin targets that trap vancomycin in the outer cell wall. In contrast, VRSA strains acquire vancomycin resistance through horizontal transfer of resistance genes from VRE, an opportunity provided in individuals coinfecting with both VRE and MRSA. VRSA exhibit a higher level of resistance, with MICs of 16 µg/mL or higher.[\[footnote\]](#) In the case of all three types of vancomycin-resistant bacteria, rapid clinical identification is necessary so proper procedures to limit spread can be implemented. The oxazolidinones like linezolid are useful for the treatment of these vancomycin-resistant, opportunistic pathogens, as well as MRSA.

Centers for Disease Control and Prevention. “Healthcare-Associated Infections (HAI): General Information about VISA/VRSA.”

http://www.cdc.gov/HAI/organisms/visa_vrsa/visa_vrsa.html. Accessed June 2, 2016.

Extended-Spectrum β -Lactamase–Producing Gram-Negative Pathogens

Gram-negative pathogens that produce **extended-spectrum β -lactamases (ESBLs)** show resistance well beyond just penicillins. The spectrum of β -lactams inactivated by ESBLs provides for resistance to all penicillins, cephalosporins, monobactams, and the β -lactamase-inhibitor combinations, but not the carbapenems. An even greater concern is that the genes encoding for ESBLs are usually found on mobile plasmids that also contain genes for resistance to other drug classes (e.g., fluoroquinolones, aminoglycosides, tetracyclines), and may be readily spread to other bacteria by horizontal gene transfer. These multidrug-resistant bacteria are members of the intestinal microbiota of some individuals, but they are also important causes of opportunistic infections in hospitalized patients, from whom they can be spread to other people.

Carbapenem-Resistant Gram-Negative Bacteria

The occurrence of **carbapenem-resistant Enterobacteriaceae (CRE)** and carbapenem resistance among other gram-negative bacteria (e.g., *P. aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*) is a

growing health-care concern. These pathogens develop resistance to carbapenems through a variety of mechanisms, including production of carbapenemases (broad-spectrum β -lactamases that inactivate all β -lactams, including carbapenems), active efflux of carbapenems out of the cell, and/or prevention of carbapenem entry through porin channels. Similar to concerns with ESBLs, carbapenem-resistant, gram-negative pathogens are usually resistant to multiple classes of antibacterials, and some have even developed pan-resistance (resistance to all available antibacterials). Infections with carbapenem-resistant, gram-negative pathogens commonly occur in health-care settings through interaction with contaminated individuals or medical devices, or as a result of surgery.

Multidrug-Resistant *Mycobacterium tuberculosis*

The emergence of **multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB)** and **extensively drug-resistant *Mycobacterium tuberculosis* (XDR-TB)** is also of significant global concern. MDR-TB strains are resistant to both rifampin and isoniazid, the drug combination typically prescribed for treatment of tuberculosis. XDR-TB strains are additionally resistant to any fluoroquinolone and at least one of three other drugs (amikacin, kanamycin, or capreomycin) used as a second line of treatment, leaving these patients very few treatment options. Both types of pathogens are particularly problematic in immunocompromised persons, including those suffering from HIV infection. The development of resistance in these strains often results from the incorrect use of antimicrobials for tuberculosis treatment, selecting for resistance.

Note:

- How does drug resistance lead to superbugs?

Note:

To learn more about the [top 18 drug-resistant threats](#) to the US, visit the CDC's website.

Note:

Factory Farming and Drug Resistance

Although animal husbandry has long been a major part of agriculture in America, the rise of concentrated animal feeding operations (CAFOs) since the 1950s has brought about some new environmental issues, including the contamination of water and air with biological waste, and ethical issues regarding animal rights also are associated with growing animals in this way. Additionally, the increase in CAFOs involves the extensive use of antimicrobial drugs in raising livestock. Antimicrobials are used to prevent the development of infectious disease in the close quarters of CAFOs; however, the majority of antimicrobials used in factory farming are for the promotion of growth—in other words, to grow larger animals.

The mechanism underlying this enhanced growth remains unclear. These antibiotics may not necessarily be the same as those used clinically for humans, but they are structurally related to drugs used for humans. As a result, use of antimicrobial drugs in animals can select for antimicrobial resistance, with these resistant bacteria becoming cross-resistant to drugs typically used in humans. For example, tylosin use in animals appears to select for bacteria also cross-resistant to other macrolides, including erythromycin, commonly used in humans.

Concentrations of the drug-resistant bacterial strains generated by CAFOs become increased in water and soil surrounding these farms. If not directly pathogenic in humans, these resistant bacteria may serve as a reservoir of mobile genetic elements that can then pass resistance genes to human pathogens. Fortunately, the cooking process typically inactivates any antimicrobials remaining in meat, so humans typically are not directly ingesting these drugs. Nevertheless, many people are calling for more judicious use of these drugs, perhaps charging farmers user fees to reduce indiscriminate use. In fact, in 2012, the FDA published guidelines for farmers who voluntarily phase out the use of antimicrobial drugs except under veterinary supervision and when necessary to ensure animal health. Although following the guidelines is voluntary at this time, the FDA does

recommend what it calls “judicious” use of antimicrobial drugs in food-producing animals in an effort to decrease antimicrobial resistance.

Note:

Part 3

Unfortunately, Marisa’s urinary tract infection did not resolve with ciprofloxacin treatment. Laboratory testing showed that her infection was caused by a strain of *Klebsiella pneumoniae* with significant antimicrobial resistance. The resistance profile of this *K. pneumoniae* included resistance to the carbapenem class of antibacterials, a group of β -lactams that is typically reserved for the treatment of highly resistant bacteria. *K. pneumoniae* is an opportunistic, capsulated, gram-negative rod that may be a member of the normal microbiota of the intestinal tract, but may also cause a number of diseases, including pneumonia and UTIs.

Specific laboratory tests looking for carbapenemase production were performed on Marisa’s samples and came back positive. Based upon this result, in combination with her health history, production of a carbapenemase known as the New Delhi Metallo- β -lactamase (NDM) was suspected. Although the origin of the NDM carbapenemase is not completely known, many patients infected with NDM-containing strains have travel histories involving hospitalizations in India or surrounding countries.

- How would doctors determine which types of antimicrobial drugs should be administered?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- **Antimicrobial resistance** is on the rise and is the result of selection of drug-resistant strains in clinical environments, the overuse and misuse

of antibacterials, the use of subtherapeutic doses of antibacterial drugs, and poor patient compliance with antibacterial drug therapies.

- Drug resistance genes are often carried on plasmids or in transposons that can undergo vertical transfer easily and between microbes through horizontal gene transfer.
- Common modes of antimicrobial drug resistance include drug modification or inactivation, prevention of cellular uptake or efflux, target modification, target overproduction or enzymatic bypass, and target mimicry.
- Problematic microbial strains showing extensive antimicrobial resistance are emerging; many of these strains can reside as members of the normal microbiota in individuals but also can cause opportunistic infection. The transmission of many of these highly resistant microbial strains often occurs in clinical settings, but can also be community-acquired.

Multiple Choice

Exercise:

Problem:

Which of the following resistance mechanisms describes the function of β -lactamase?

- A. efflux pump
- B. target mimicry
- C. drug inactivation
- D. target overproduction

Solution:

C

Exercise:

Problem:

Which of the following resistance mechanisms is commonly effective against a wide range of antimicrobials in multiple classes?

- A. efflux pump
- B. target mimicry
- C. target modification
- D. target overproduction

Solution:

A

Exercise:**Problem:**

Which of the following resistance mechanisms is the most nonspecific to a particular class of antimicrobials?

- A. drug modification
- B. target mimicry
- C. target modification
- D. efflux pump

Solution:

D

Exercise:**Problem:**

Which of the following types of drug-resistant bacteria do not typically persist in individuals as a member of their intestinal microbiota?

- A. MRSA

- B. VRE
- C. CRE
- D. ESBL-producing bacteria

Solution:

A

Fill in the Blank

Exercise:

Problem:

Staphylococcus aureus, including MRSA strains, may commonly be carried as a normal member of the _____ microbiota in some people.

Solution:

nasal

Short Answer

Exercise:

Problem:

Why does the length of time of antimicrobial treatment for tuberculosis contribute to the rise of resistant strains?

Exercise:

Problem:

What is the difference between multidrug resistance and cross-resistance?

LEARNING OBJECTIVES

- Describe the methods and strategies used for discovery of new antimicrobial agents.

With the continued evolution and spread of antimicrobial resistance, and now the identification of pan-resistant bacterial pathogens, the search for new antimicrobials is essential for preventing the postantibiotic era. Although development of more effective semisynthetic derivatives is one strategy, resistance to them develops rapidly because bacterial pathogens are already resistant to earlier-generation drugs in the family and can easily mutate and develop resistance to the new semisynthetic drugs. Today, scientists continue to hunt for new antimicrobial compounds and explore new avenues of antimicrobial discovery and synthesis. They check large numbers of soils and microbial products for antimicrobial activity by using high-throughput screening methods, which use automation to test large numbers of samples simultaneously. The recent development of the iChip^[footnote] allows researchers to investigate the antimicrobial-producing capabilities of soil microbes that are difficult to grow by standard cultivation techniques in the laboratory. Rather than grow the microbes in the laboratory, they are grown in situ—right in the soil. Use of the iChip has resulted in the discovery of teixobactin, a novel antimicrobial from Mount Ararat, Turkey. Teixobactin targets two distinct steps in gram-positive cell wall synthesis and for which antimicrobial resistance appears not yet to have evolved.

L. Losee et al. “A New Antibiotic Kills Pathogens Without Detectable Resistance.” *Nature* 517 no. 7535 (2015):455–459.

Although soils have been widely examined, other environmental niches have not been tested as fully. Since 70% of the earth is covered with water, marine environments could be mined more fully for the presence of antimicrobial-producing microbes. In addition, researchers are using combinatorial chemistry, a method for making a very large number of related compounds from simple precursors, and testing them for antimicrobial activity. An additional strategy that needs to be explored further is the development of compounds that inhibit resistance mechanisms and restore the activity of older drugs, such as the strategy described earlier for β -lactamase inhibitors like clavulanic acid. Finally, developing inhibitors of virulence factor production and function could be a very important avenue. Although this strategy would not be directly antibacterial, drugs that slow the progression of an infection could provide an advantage for the immune system and could be used successfully in combination with antimicrobial drugs.

Note:

- What are new sources and strategies for developing drugs to fight infectious diseases?

Note:

The (Free?) Market for New Antimicrobials

There used to be plenty of antimicrobial drugs on the market to treat infectious diseases. However, the spread of antimicrobial resistance has created a need for new antibiotics to replace those that are no longer as effective as they once were. Unfortunately, pharmaceutical companies are not particularly motivated to fill this need. As of 2009, all but five pharmaceutical companies had moved away from antimicrobial drug development. [\[footnote\]](#) As a result, the number of FDA approvals of new antimicrobials has fallen drastically in recent decades ([\[link\]](#)).

H.W. Boucher et al. “Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America.” *Clinical Infectious Diseases*

48 no. 1 (2009):1–12.

Given that demand usually encourages supply, one might expect pharmaceutical companies to be rushing to get back in the business of developing new antibiotics. But developing new drugs is a lengthy process and requires large investments in research and development.

Pharmaceutical companies can typically get a higher return on their investment by developing products for chronic, nonmicrobial diseases like diabetes; such drugs must be taken for life, and therefore generate more long-term revenue than an antibiotic that does its job in a week or two. But what will happen when drugs like vancomycin, a superantimicrobial reserved for use as a last resort, begin to lose their effectiveness against ever more drug-resistant superbugs? Will drug companies wait until all antibiotics have become useless before beginning to look for new ones?

Recently, it has been suggested that large pharmaceutical companies should be given financial incentives to pursue such research. In September 2014, the White House released an executive order entitled “Combating Antibiotic Resistant Bacteria,” calling upon various government agencies and the private sector to work together to “accelerate basic and applied research and development for new antimicrobials, other therapeutics, and vaccines.”[\[footnote\]](#) As a result, as of March 2015, President Obama’s

proposed fiscal year 2016 budget doubled the amount of federal funding to \$1.2 billion for “combating and preventing antibiotic resistance,” which includes money for antimicrobial research and development.[\[footnote\]](#)

Similar suggestions have also been made on a global scale. In December 2014, a report chaired by former Goldman Sachs economist Jim O’Neill was published in *The Review on Antimicrobial Resistance*.[\[footnote\]](#)

The White House. *National Action Plan for Combating Antibiotic-Resistant Bacteria*. Washington, DC: The White House, 2015.

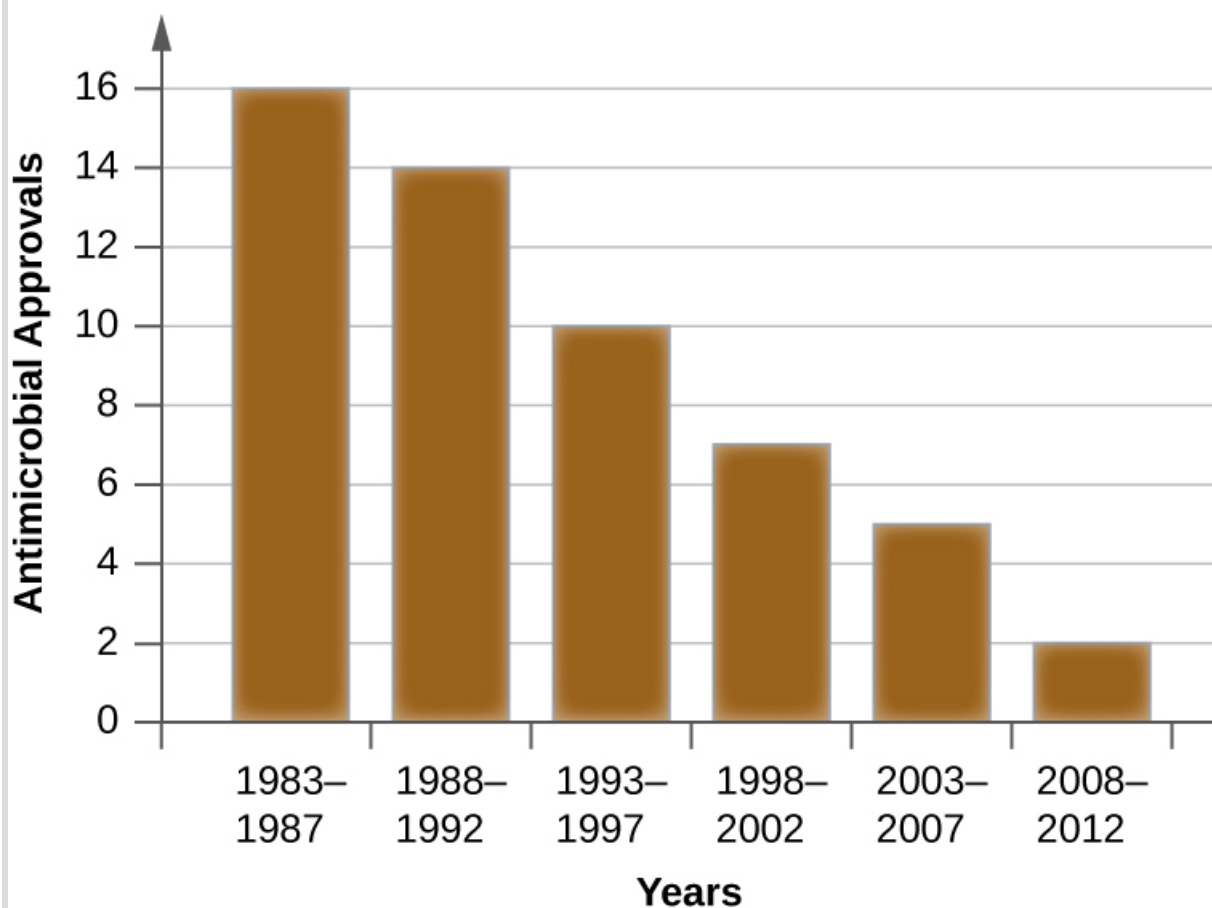
White House Office of the Press Secretary. “Fact Sheet: Obama Administration Releases National Action Plan to Combat Antibiotic-Resistant Bacteria.” March 27, 2015. <https://www.whitehouse.gov/the-press-office/2015/03/27/fact-sheet-obama-administration-releases-national-action-plan-combat-ant>

Review on Antimicrobial Resistance. <http://amr-review.org>. Accessed June 1, 2016.

These developments reflect the growing belief that for-profit pharmaceutical companies must be subsidized to encourage development

of new antimicrobials. But some ask whether pharmaceutical development should be motivated by profit at all. Given that millions of lives may hang in the balance, some might argue that drug companies have an ethical obligation to devote their research and development efforts to high-utility drugs, as opposed to highly profitable ones. Yet this obligation conflicts with the fundamental goals of a for-profit company. Are government subsidies enough to ensure that drug companies make the public interest a priority, or should government agencies assume responsibility for developing critical drugs that may have little or no return on investment?

New Antimicrobials Approved by FDA, 1983–2012



In recent decades, approvals of new antimicrobials by the FDA have steadily fallen. In the five-year period from 1983–1987, 16 new antimicrobial drugs were approved, compared to just two from 2008–2012.

Note:

To further examine the scope of the problem, view [this](#) video.

To [learn more](#) about the history of antimicrobial drug discovery, visit Michigan State University's Antimicrobial Resistance Learning Site.

Key Concepts and Summary

- Current research into the development of antimicrobial drugs involves the use of high-throughput screening and combinatorial chemistry technologies.
- New technologies are being developed to discover novel antibiotics from soil microorganisms that cannot be cultured by standard laboratory methods.
- Additional strategies include searching for antibiotics from sources other than soil, identifying new antibacterial targets, using combinatorial chemistry to develop novel drugs, developing drugs that inhibit resistance mechanisms, and developing drugs that target virulence factors and hold infections in check.

Multiple Choice

Exercise:

Problem:

Which of the following has yielded compounds with the most antimicrobial activity?

- A. water
- B. air
- C. volcanoes
- D. soil

Solution:

D

True/False

Exercise:

Problem:

The rate of discovery of antimicrobial drugs has decreased significantly in recent decades.

Solution:

true

Critical Thinking

Exercise:

Problem:

Who should be responsible for discovering and developing new antibiotics? Support your answer with reasoning.

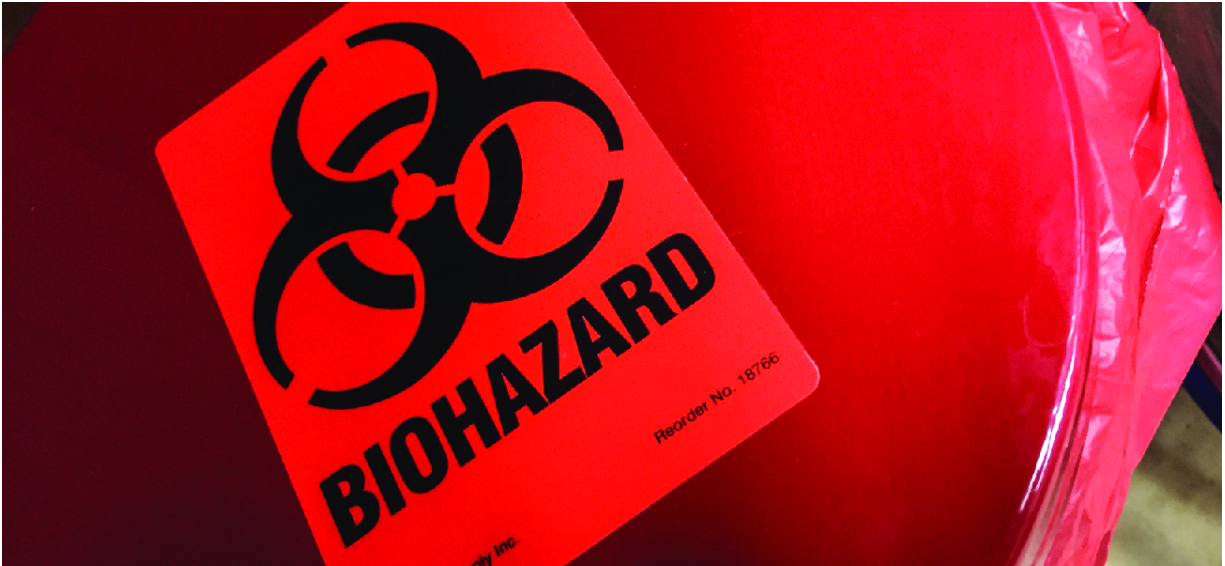
Introduction

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Signs like
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spread.

Microbiology
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the field of
epidemiology
, which
focuses on
containing
the spread of
disease.

(credit:
modification
of work by
Tony
Webster)



In the United States and other developed nations, public health is a key function of government. A healthy citizenry is more productive, content, and prosperous; high rates of death and disease, on the other hand, can severely hamper economic productivity and foster social and political instability. The burden of disease makes it difficult for citizens to work consistently, maintain employment, and accumulate wealth to better their lives and support a growing economy.

In this chapter, we will explore the intersections between microbiology and epidemiology, the science that underlies public health. Epidemiology studies how disease originates and spreads throughout a population, with the goal of preventing outbreaks and containing them when they do occur. Over the past two centuries, discoveries in epidemiology have led to public health policies that have transformed life in developed nations, leading to the eradication (or near eradication) of many diseases that were once causes of great human suffering and premature death. However, the work of epidemiologists is far from finished. Numerous diseases continue to plague humanity, and new diseases are always emerging. Moreover, in the developing world, lack of infrastructure continues to pose many challenges to efforts to contain disease.

The Language of Epidemiologists

LEARNING OBJECTIVES

- Explain the difference between prevalence and incidence of disease
- Distinguish the characteristics of sporadic, endemic, epidemic, and pandemic diseases
- Explain the use of Koch's postulates and their modifications to determine the etiology of disease
- Explain the relationship between epidemiology and public health

Note:

Part 1

In late November and early December, a hospital in western Florida started to see a spike in the number of cases of acute gastroenteritis-like symptoms. Patients began arriving at the emergency department complaining of excessive bouts of emesis (vomiting) and diarrhea (with no blood in the stool). They also complained of abdominal pain and cramping, and most were severely dehydrated. Alarmed by the number of cases, hospital staff made some calls and learned that other regional hospitals were also seeing 10 to 20 similar cases per day.

- What are some possible causes of this outbreak?
- In what ways could these cases be linked, and how could any suspected links be confirmed?

Jump to the [next](#) Clinical Focus box.

The field of **epidemiology** concerns the geographical distribution and timing of infectious disease occurrences and how they are transmitted and maintained in nature, with the goal of recognizing and controlling outbreaks. The science of epidemiology includes **etiology** (the study of the causes of disease) and investigation of disease transmission (mechanisms by which a disease is spread).

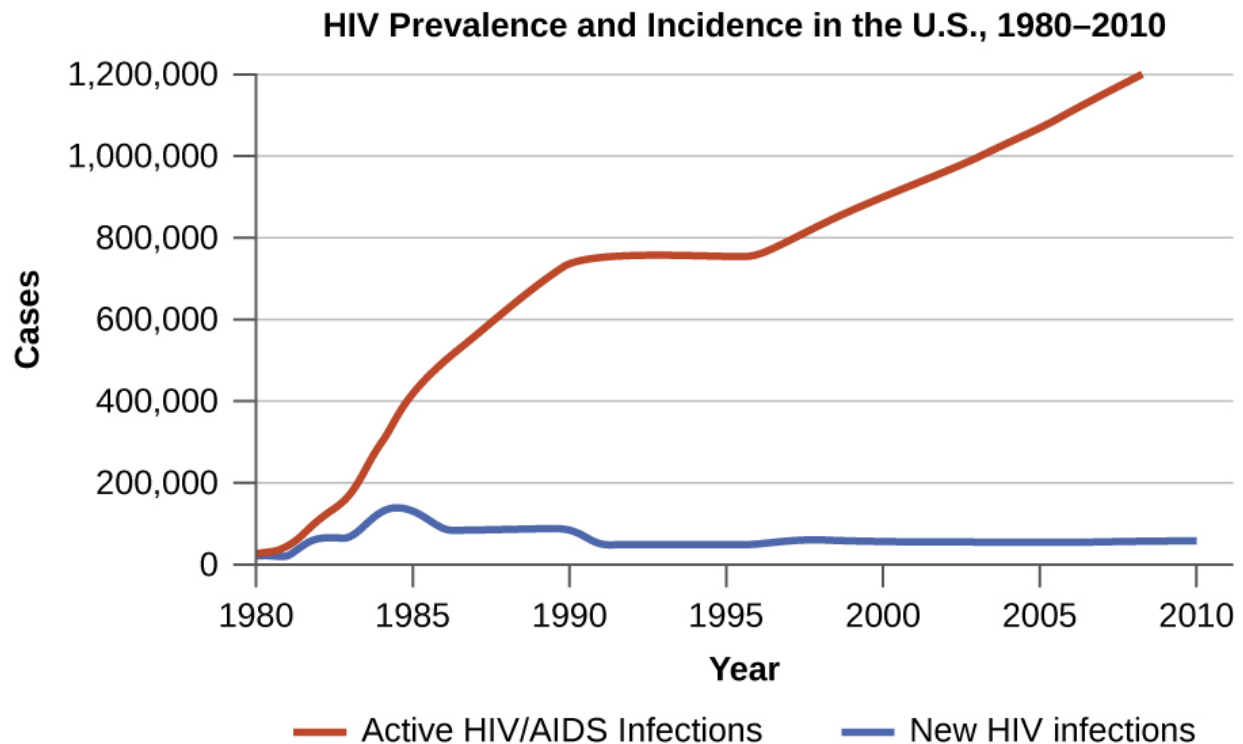
Analyzing Disease in a Population

Epidemiological analyses are always carried out with reference to a population, which is the group of individuals that are at risk for the disease or condition. The population can be defined geographically, but if only a portion of the individuals in that area are susceptible, additional criteria may be required. Susceptible individuals may be defined by particular behaviors, such as intravenous drug use, owning particular pets, or membership in an institution, such as a college. Being able to define the population is important because most measures of interest in epidemiology are made with reference to the size of the population.

The state of being diseased is called **morbidity**. Morbidity in a population can be expressed in a few different ways. Morbidity or total morbidity is expressed in numbers of individuals without reference to the size of the population. The **morbidity rate** can be expressed as the number of diseased individuals out of a standard number of individuals in the population, such as 100,000, or as a percent of the population.

There are two aspects of morbidity that are relevant to an epidemiologist: a disease's **prevalence** and its **incidence**. Prevalence is the number, or proportion, of individuals with a particular illness in a given population at a point in time. For example, the Centers for Disease Control and Prevention (CDC) estimated that in 2012, there were about 1.2 million people 13 years and older with an active human immunodeficiency virus (HIV) infection. Expressed as a proportion, or rate, this is a prevalence of 467 infected persons per 100,000 in the population.[\[footnote\]](#) On the other hand, incidence is the number or proportion of *new* cases in a period of time. For the same year and population, the CDC estimates that there were 43,165 newly diagnosed cases of HIV infection, which is an incidence of 13.7 new cases per 100,000 in the population.[\[footnote\]](#) The relationship between incidence and prevalence can be seen in [\[link\]](#). For a chronic disease like HIV infection, prevalence will generally be higher than incidence because it represents the cumulative number of new cases over many years minus the number of cases that are no longer active (e.g., because the patient died or was cured). H. Irene Hall, Qian An, Tian Tang, Ruiguang Song, Mi Chen, Timothy Green, and Jian Kang. "Prevalence of Diagnosed and Undiagnosed HIV Infection—United States, 2008–2012." *Morbidity and Mortality Weekly Report* 64, no. 24 (2015): 657–662. Centers for Disease Control and Prevention. "Diagnoses of HIV Infection in the United States and Dependent Areas, 2014." *HIV Surveillance Report* 26 (2015).

In addition to morbidity rates, the incidence and prevalence of **mortality** (death) may also be reported. A mortality rate can be expressed as the percentage of the population that has died from a disease or as the number of deaths per 100,000 persons (or other suitable standard number).



This graph compares the incidence of HIV (the number of new cases reported each year) with the prevalence (the total number of cases each year). Prevalence and incidence can also be expressed as a rate or proportion for a given population.

Note:

- Explain the difference between incidence and prevalence.
- Describe how morbidity and mortality rates are expressed.

Patterns of Incidence

Diseases that are seen only occasionally, and usually without geographic concentration, are called **sporadic diseases**. Examples of sporadic diseases include tetanus, rabies, and plague. In the United States, *Clostridium tetani*, the bacterium that causes tetanus, is ubiquitous in the soil environment, but incidences of infection occur only rarely and in scattered locations because most individuals are vaccinated, clean wounds appropriately, or are only rarely in a situation that would cause infection.[\[footnote\]](#)

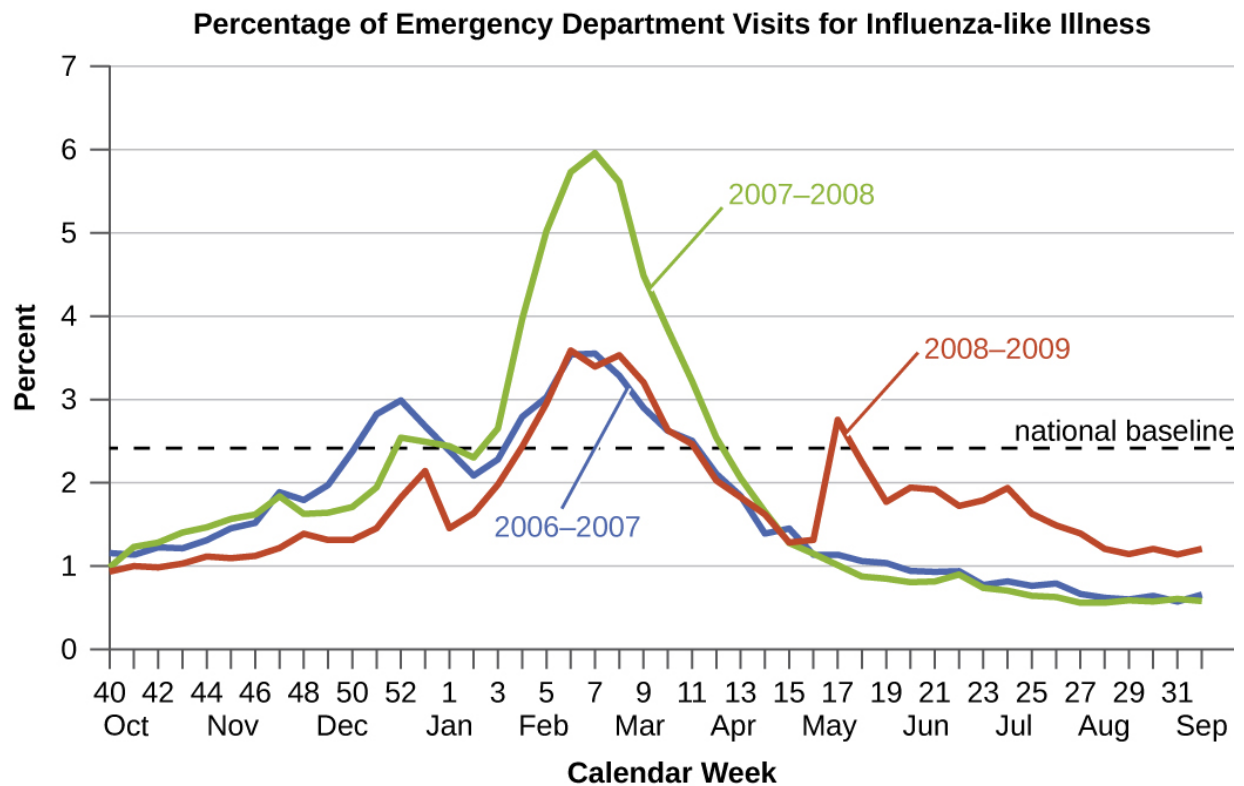
Likewise in the United States there are a few scattered cases of plague each year, usually contracted from rodents in rural areas in the western states.[\[footnote\]](#) Centers for Disease Control and Prevention. “Tetanus Surveillance—United States, 2001–2008.” *Morbidity and Mortality Weekly Report* 60, no. 12 (2011): 365. Centers for Disease Control and Prevention. “Plague in the United States.” 2015. <http://www.cdc.gov/plague/maps>. Accessed June 1, 2016.

Diseases that are constantly present (often at a low level) in a population within a particular geographic region are called **endemic diseases**. For example, malaria is endemic to some regions of Brazil, but is not endemic to the United States.

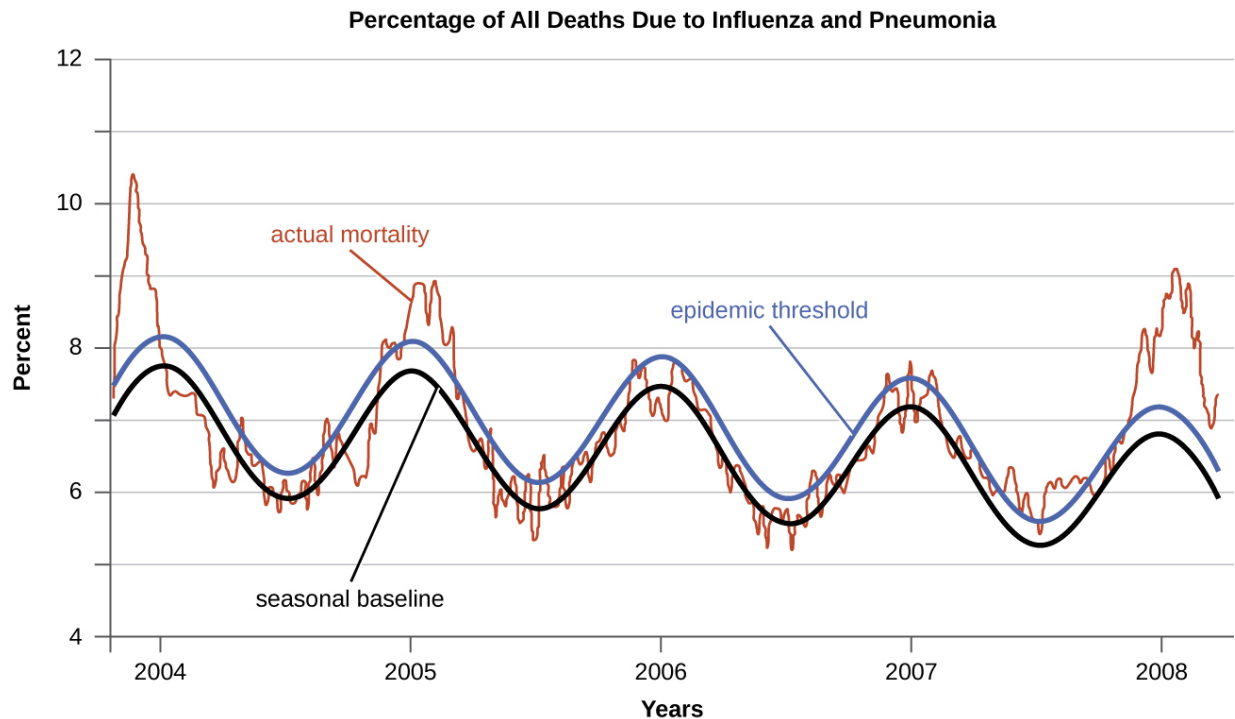
Diseases for which a larger than expected number of cases occurs in a short time within a geographic region are called **epidemic diseases**. Influenza is a good example of a commonly epidemic disease. Incidence patterns of influenza tend to rise each winter in the northern hemisphere. These seasonal increases are expected, so it would not be accurate to say that influenza is epidemic every winter; however, some winters have an unusually large number of seasonal influenza cases in particular regions, and such situations would qualify as epidemics ([\[link\]](#) and [\[link\]](#)).

An epidemic disease signals the breakdown of an equilibrium in disease frequency, often resulting from some change in environmental conditions or in the population. In the case of influenza, the disruption can be due to antigenic shift or drift (see [Virulence Factors of Bacterial and Viral Pathogens](#)), which allows influenza virus strains to circumvent the acquired immunity of their human hosts.

An epidemic that occurs on a worldwide scale is called a **pandemic disease**. For example, HIV/AIDS is a pandemic disease and novel influenza virus strains often become pandemic.



The 2007–2008 influenza season in the United States saw much higher than normal numbers of visits to emergency departments for influenza-like symptoms as compared to the previous and the following years. (credit: modification of work by Centers for Disease Control and Prevention)



The seasonal epidemic threshold (blue curve) is set by the CDC-based data from the previous five years. When actual mortality rates exceed this threshold, a disease is considered to be epidemic. As this graph shows, pneumonia- and influenza-related mortality saw pronounced epidemics during the winters of 2003–2004, 2005, and 2008. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- Explain the difference between sporadic and endemic disease.
- Explain the difference between endemic and epidemic disease.

Note:

Part 2

Hospital physicians suspected that some type of food poisoning was to blame for the sudden post-Thanksgiving outbreak of gastroenteritis in western Florida. Over a two-week period, 254 cases were observed, but by the end of the first week of December,

the epidemic ceased just as quickly as it had started. Suspecting a link between the cases based on the localized nature of the outbreak, hospitals handed over their medical records to the regional public health office for study.

Laboratory testing of stool samples had indicated that the infections were caused by *Salmonella* bacteria. Patients ranged from children as young as three to seniors in their late eighties. Cases were nearly evenly split between males and females. Across the region, there had been three confirmed deaths in the outbreak, all due to severe dehydration. In each of the fatal cases, the patients had not sought medical care until their symptoms were severe; also, all of the deceased had preexisting medical conditions such as congestive heart failure, diabetes, or high blood pressure.

After reviewing the medical records, epidemiologists with the public health office decided to conduct interviews with a randomly selected sample of patients.

- What conclusions, if any, can be drawn from the medical records?
- What would epidemiologists hope to learn by interviewing patients? What kinds of questions might they ask?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Etiology

When studying an epidemic, an epidemiologist's first task is to determinate the cause of the disease, called the **etiologic agent** or **causative agent**. Connecting a disease to a specific pathogen can be challenging because of the extra effort typically required to demonstrate direct causation as opposed to a simple association. It is not enough to observe an association between a disease and a suspected pathogen; controlled experiments are needed to eliminate other possible causes. In addition, pathogens are typically difficult to detect when there is no immediate clue as to what is causing the outbreak. Signs and symptoms of disease are also commonly nonspecific, meaning that many different agents can give rise to the same set of signs and symptoms. This complicates diagnosis even when a causative agent is familiar to scientists.

Robert Koch was the first scientist to specifically demonstrate the causative agent of a disease (anthrax) in the late 1800s. Koch developed four criteria, now known as Koch's postulates, which had to be met in order to positively link a disease with a pathogenic microbe. Without Koch's postulates, the Golden Age of Microbiology would not have occurred. Between 1876 and 1905, many common diseases were linked with their etiologic agents, including cholera, diphtheria, gonorrhea, meningitis, plague, syphilis, tetanus, and tuberculosis. Today, we use the molecular Koch's postulates, a variation of Koch's original postulates that can be used to establish a link between the disease state and virulence traits unique to a pathogenic strain of a microbe. Koch's original

postulates and molecular Koch's postulates were described in more detail in [How Pathogens Cause Disease](#).

Note:

- List some challenges to determining the causative agent of a disease outbreak.

The Role of Public Health Organizations

The main national public health agency in the United States is the **Centers for Disease Control and Prevention (CDC)**, an agency of the Department of Health and Human Services. The CDC is charged with protecting the public from disease and injury. One way that the CDC carries out this mission is by overseeing the National Notifiable Disease Surveillance System (NNDSS) in cooperation with regional, state, and territorial public health departments. The NNDSS monitors diseases considered to be of public health importance on a national scale. Such diseases are called **notifiable diseases** or **reportable diseases** because all cases must be reported to the CDC. A physician treating a patient with a notifiable disease is legally required to submit a report on the case. Notifiable diseases include HIV infection, measles, West Nile virus infections, and many others. Some states have their own lists of notifiable diseases that include diseases beyond those on the CDC's list.

Notifiable diseases are tracked by epidemiological studies and the data is used to inform health-care providers and the public about possible risks. The CDC publishes the ***Morbidity and Mortality Weekly Report (MMWR)***, which provides physicians and health-care workers with updates on public health issues and the latest data pertaining to notifiable diseases. [\[link\]](#) is an example of the kind of data contained in the *MMWR*.

**Incidence of Four Notifiable Diseases in the United States, Week Ending
January 2, 2016**

Disease	Current Week (Jan 2, 2016)	Median of Previous 52 Weeks	Maximum of Previous 52 Weeks	Cumulative Cases 2015
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Campylobacteriosis	406	869	1,385	46,618
<i>Chlamydia trachomatis</i> infection	11,024	28,562	31,089	1,425,303
Giardiasis	115	230	335	11,870
Gonorrhea	3,207	7,155	8,283	369,926

Note:

The current [Morbidity and Mortality Weekly Report](#) is available online.

Note:

- Describe how health agencies obtain data about the incidence of diseases of public health importance.

Key Concepts and Summary

- **Epidemiology** is the science underlying public health.
- **Morbidity** means being in a state of illness, whereas **mortality** refers to death; both **morbidity rates** and **mortality rates** are of interest to epidemiologists.
- **Incidence** is the number of new cases (morbidity or mortality), usually expressed as a proportion, during a specified time period; **prevalence** is the total number affected in the population, again usually expressed as a proportion.

- **Sporadic diseases** only occur rarely and largely without a geographic focus. **Endemic diseases** occur at a constant (and often low) level within a population. **Epidemic diseases** and **pandemic diseases** occur when an outbreak occurs on a significantly larger than expected level, either locally or globally, respectively.
- **Koch's postulates** specify the procedure for confirming a particular pathogen as the etiologic agent of a particular disease. Koch's postulates have limitations in application if the microbe cannot be isolated and cultured or if there is no animal host for the microbe. In this case, molecular Koch's postulates would be utilized.
- In the United States, the **Centers for Disease Control and Prevention** monitors **notifiable diseases** and publishes weekly updates in the *Morbidity and Mortality Weekly Report*.

Matching

Exercise:

Problem: Match each term with its description.

___sporadic disease	A. the number of disease cases per 100,000 individuals
___endemic disease	B. a disease in higher than expected numbers around the world
___pandemic disease	C. the number of deaths from a disease for every 10,000 individuals
___morbidity rate	D. a disease found occasionally in a region with cases occurring mainly in isolation from each other
___mortality rate	E. a disease found regularly in a region

Solution:

D, E, B, A, C

Fill in the Blank

Exercise:

Problem:

The _____ collects data and conducts epidemiologic studies in the United States.

Solution:

Centers for Disease Control and Prevention, or CDC

Short Answer

Exercise:

Problem:

During an epidemic, why might the prevalence of a disease at a particular time not be equal to the sum of the incidences of the disease?

Exercise:

Problem:

In what publication would you find data on emerging/reemerging diseases in the United States?

Critical Thinking

Exercise:

Problem:

Why might an epidemiological population in a state not be the same size as the number of people in a state? Use an example.

LEARNING OBJECTIVES

- Explain the research approaches used by the pioneers of epidemiology
- Explain how descriptive, analytical, and experimental epidemiological studies go about determining the cause of morbidity and mortality

Epidemiology has its roots in the work of physicians who looked for patterns in disease occurrence as a way to understand how to prevent it. The idea that disease could be transmitted was an important precursor to making sense of some of the patterns. In 1546, Girolamo Fracastoro first proposed the germ theory of disease in his essay *De Contagione et Contagiosis Morbis*, but this theory remained in competition with other theories, such as the miasma hypothesis, for many years (see [What Our Ancestors Knew](#)). Uncertainty about the cause of disease was not an absolute barrier to obtaining useful knowledge from patterns of disease. Some important researchers, such as Florence Nightingale, subscribed to the miasma hypothesis. The transition to acceptance of the germ theory during the 19th century provided a solid mechanistic grounding to the study of disease patterns. The studies of 19th century physicians and researchers such as John Snow, Florence Nightingale, Ignaz Semmelweis, Joseph Lister, Robert Koch, Louis Pasteur, and others sowed the seeds of modern epidemiology.

Pioneers of Epidemiology

John Snow ([\[link\]](#)) was a British physician known as the father of epidemiology for determining the source of the 1854 Broad Street cholera epidemic in London. Based on observations he had made during an earlier cholera outbreak (1848–1849), Snow proposed that cholera was spread through a fecal-oral route of transmission and that a microbe was the infectious agent. He investigated the 1854 cholera epidemic in two ways. First, suspecting that contaminated water was the source of the epidemic, Snow identified the source of water for those infected. He found a high frequency of cholera cases among individuals who obtained their water from the River Thames downstream from London. This water contained the refuse and sewage from London and settlements upstream. He also noted that brewery workers did not contract cholera and on investigation found the owners provided the workers with beer to drink and stated that they likely did not drink water.[\[footnote\]](#) Second, he also painstakingly mapped the incidence of cholera and found a high frequency among those individuals using a particular water pump located on Broad Street. In response to Snow's advice, local officials removed the pump's handle, [\[footnote\]](#) resulting in the containment of the Broad Street cholera epidemic.

John Snow. *On the Mode of Communication of Cholera. Second edition, Much Enlarged*. John Churchill, 1855.

John Snow. "The Cholera near Golden-Wquare, and at Deptford." *Medical Times and Gazette* 9 (1854): 321–322.

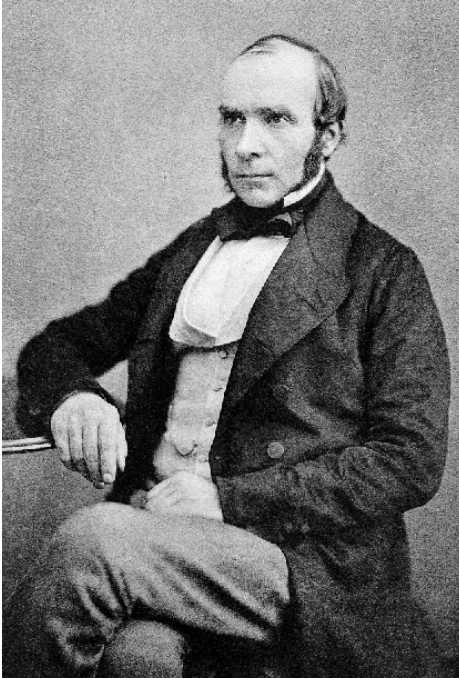
<http://www.ph.ucla.edu/epi/snow/choleragoldensquare.html>.

Snow's work represents an early epidemiological study and it resulted in the first known public health response to an epidemic. Snow's meticulous case-tracking methods are now common practice in studying disease outbreaks and in associating new diseases with their causes. His work further shed light on unsanitary sewage practices and the effects of waste dumping in the Thames. Additionally, his work supported the germ theory of disease, which argued disease could be transmitted through contaminated items, including water contaminated with fecal matter.

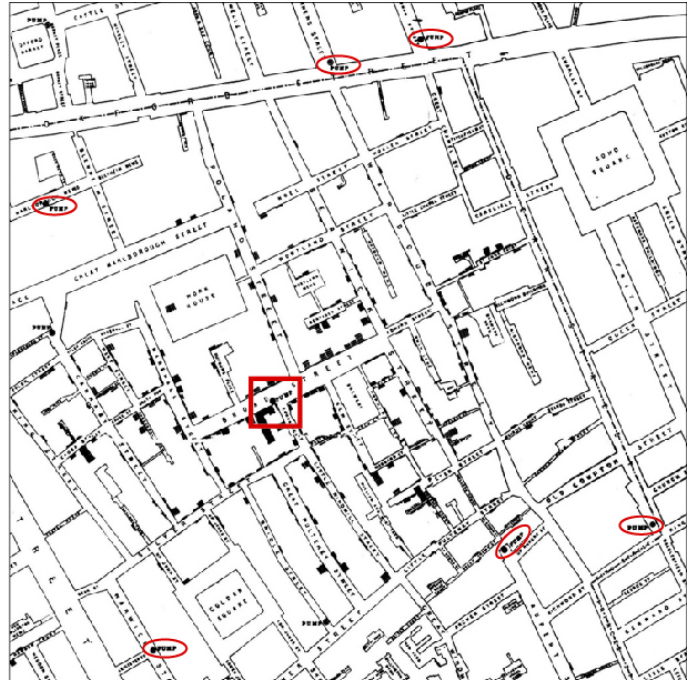
Snow's work illustrated what is referred to today as a **common source spread** of infectious disease, in which there is a single source for all of the individuals infected. In this case, the single source was the contaminated

well below the Broad Street pump. Types of common source spread include point source spread, continuous common source spread, and intermittent common source spread. In **point source spread** of infectious disease, the common source operates for a short time period—less than the incubation period of the pathogen. An example of point source spread is a single contaminated potato salad at a group picnic. In **continuous common source spread**, the infection occurs for an extended period of time, longer than the incubation period. An example of continuous common source spread would be the source of London water taken downstream of the city, which was continuously contaminated with sewage from upstream. Finally, with **intermittent common source spread**, infections occur for a period, stop, and then begin again. This might be seen in infections from a well that was contaminated only after large rainfalls and that cleared itself of contamination after a short period.

In contrast to common source spread, **propagated spread** occurs through direct or indirect person-to-person contact. With propagated spread, there is no single source for infection; each infected individual becomes a source for one or more subsequent infections. With propagated spread, unless the spread is stopped immediately, infections occur for longer than the incubation period. Although point sources often lead to large-scale but localized outbreaks of short duration, propagated spread typically results in longer duration outbreaks that can vary from small to large, depending on the population and the disease ([\[link\]](#)). In addition, because of person-to-person transmission, propagated spread cannot be easily stopped at a single source like point source spread.

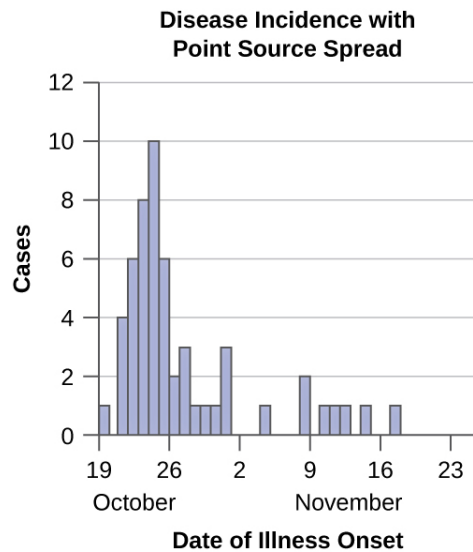


(a)

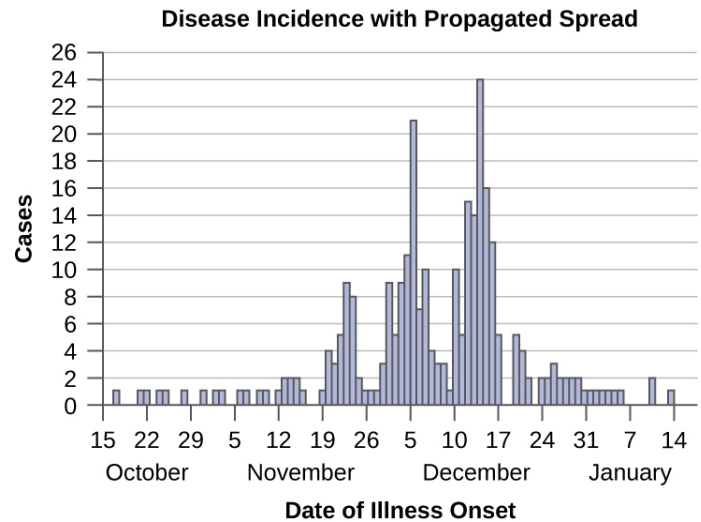


(b)

(a) John Snow (1813–1858), British physician and father of epidemiology. (b) Snow's detailed mapping of cholera incidence led to the discovery of the contaminated water pump on Broad street (red square) responsible for the 1854 cholera epidemic. (credit a: modification of work by "Rsabbatini"/Wikimedia Commons)



(a)



(b)

(a) Outbreaks that can be attributed to point source spread often have a short duration. (b) Outbreaks attributed to propagated spread can have a more extended duration. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Florence Nightingale's work is another example of an early epidemiological study. In 1854, Nightingale was part of a contingent of nurses dispatched by the British military to care for wounded soldiers during the Crimean War. Nightingale kept meticulous records regarding the causes of illness and death during the war. Her recordkeeping was a fundamental task of what would later become the science of epidemiology. Her analysis of the data she collected was published in 1858. In this book, she presented monthly frequency data on causes of death in a wedge chart histogram ([link](#)). This graphical presentation of data, unusual at the time, powerfully illustrated that the vast majority of casualties during the war occurred not due to wounds sustained in action but to what Nightingale deemed preventable infectious diseases. Often these diseases occurred because of poor sanitation and lack of access to hospital facilities. Nightingale's findings led to many reforms in the British military's system of medical care.

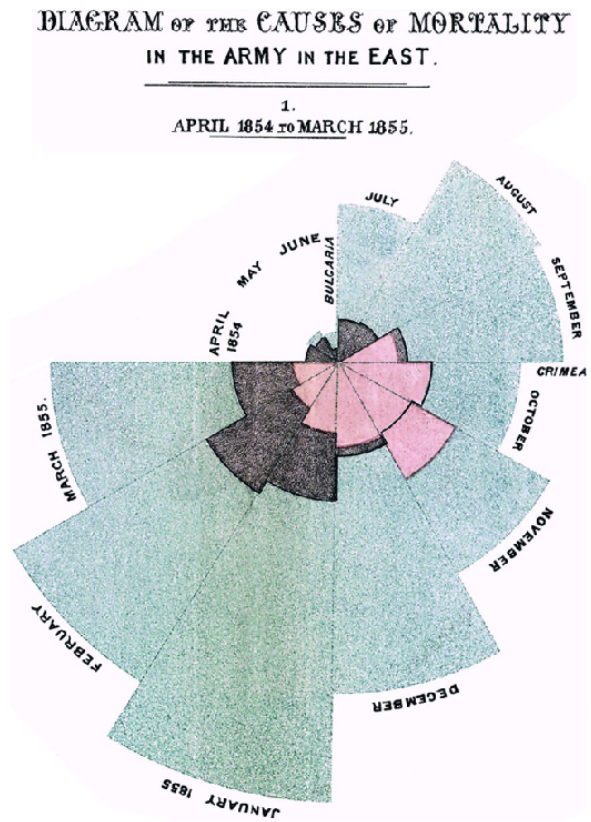
Joseph Lister provided early epidemiological evidence leading to good public health practices in clinics and hospitals. These settings were notorious in the mid-1800s for fatal infections of surgical wounds at a time when the germ theory of disease was not yet widely accepted (see [Foundations of Modern Cell Theory](#)). Most physicians did not wash their hands between patient visits or clean and sterilize their surgical tools. Lister, however, discovered the disinfecting properties of carbolic acid, also known as phenol (see [Using Chemicals to Control Microorganisms](#)). He introduced several disinfection protocols that dramatically lowered post-surgical infection rates.[\[footnote\]](#) He demanded that surgeons who worked for him use a 5% carbolic acid solution to clean their surgical tools between patients, and even went so far as to spray the solution onto bandages and over the surgical site during operations ([\[link\]](#)). He also took precautions not to introduce sources of infection from his skin or clothing by removing his coat, rolling up his sleeves, and washing his hands in a dilute solution of carbolic acid before and during the surgery.

O.M. Lidwell. “Joseph Lister and Infection from the Air.” *Epidemiology and Infection* 99 (1987): 569–578.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2249236/pdf/epidinfec00006-0004.pdf>.

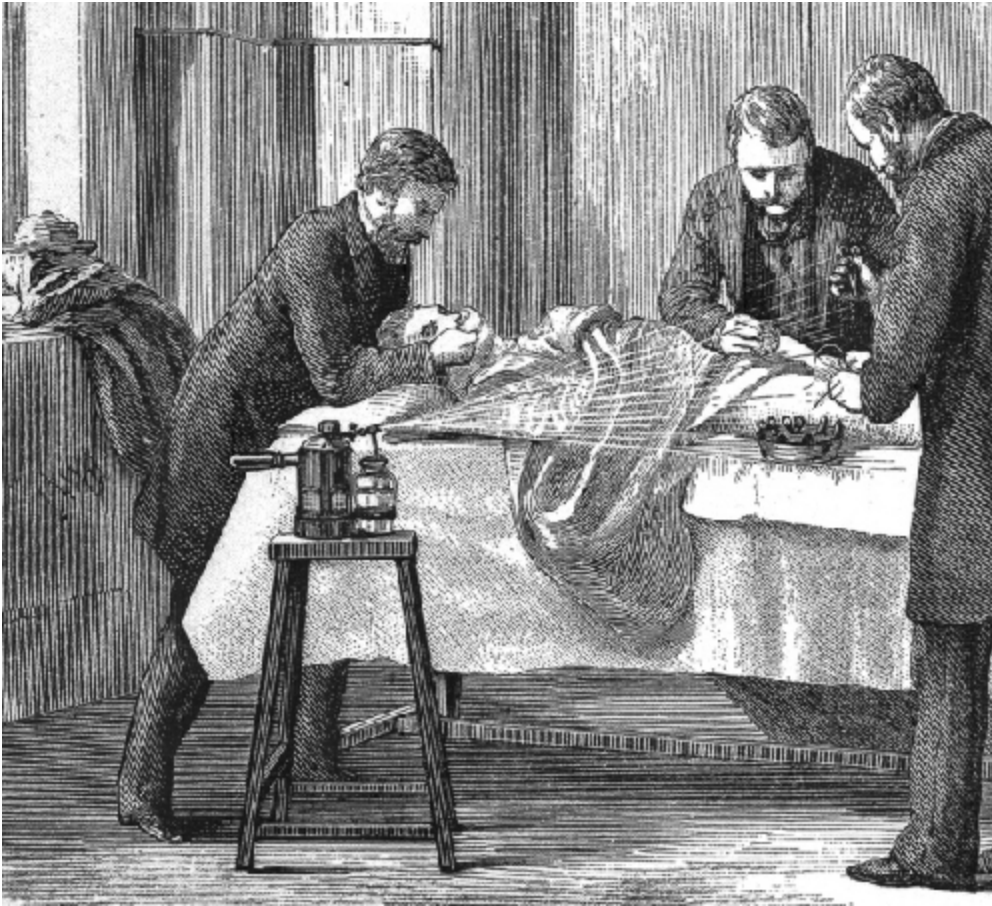


(a)



(b)

(a) Florence Nightingale reported on the data she collected as a nurse in the Crimean War. (b) Nightingale's diagram shows the number of fatalities in soldiers by month of the conflict from various causes. The total number dead in a particular month is equal to the area of the wedge for that month. The colored sections of the wedge represent different causes of death: wounds (pink), preventable infectious diseases (gray), and all other causes (brown).



Joseph Lister initiated the use of a carbolic acid (phenol) during surgeries. This illustration of a surgery shows a pressurized canister of carbolic acid being sprayed over the surgical site.

Note:

Visit the [website](#) for *The Ghost Map*, a book about Snow's work related to the Broad Street pump cholera outbreak.

John Snow's [own account of his work](#) has additional links and information. This [CDC resource](#) further breaks down the pattern expected from a point-source outbreak.

Learn more about [Nightingale's wedge chart](#) here.

Note:

- Explain the difference between common source spread and propagated spread of disease.
- Describe how the observations of John Snow, Florence Nightingale, and Joseph Lister led to improvements in public health.

Types of Epidemiological Studies

Today, epidemiologists make use of study designs, the manner in which data are gathered to test a hypothesis, similar to those of researchers studying other phenomena that occur in populations. These approaches can be divided into observational studies (in which subjects are not manipulated) and experimental studies (in which subjects are manipulated). Collectively, these studies give modern-day epidemiologists multiple tools for exploring the connections between infectious diseases and the populations of susceptible individuals they might infect.

Observational Studies

In an **observational study**, data are gathered from study participants through measurements (such as physiological variables like white blood cell count), or answers to questions in interviews (such as recent travel or exercise frequency). The subjects in an observational study are typically chosen at random from a population of affected or unaffected individuals. However, the subjects in an observational study are in no way manipulated by the researcher. Observational studies are typically easier to carry out than experimental studies, and in certain situations they may be the only studies possible for ethical reasons.

Observational studies are only able to measure associations between disease occurrence and possible causative agents; they do not necessarily prove a causal relationship. For example, suppose a study finds an association

between heavy coffee drinking and lower incidence of skin cancer. This might suggest that coffee prevents skin cancer, but there may be another unmeasured factor involved, such as the amount of sun exposure the participants receive. If it turns out that coffee drinkers work more in offices and spend less time outside in the sun than those who drink less coffee, then it may be possible that the lower rate of skin cancer is due to less sun exposure, not to coffee consumption. The observational study cannot distinguish between these two potential causes.

There are several useful approaches in observational studies. These include methods classified as descriptive epidemiology and analytical epidemiology. **Descriptive epidemiology** gathers information about a disease outbreak, the affected individuals, and how the disease has spread over time in an exploratory stage of study. This type of study will involve interviews with patients, their contacts, and their family members; examination of samples and medical records; and even histories of food and beverages consumed. Such a study might be conducted while the outbreak is still occurring. Descriptive studies might form the basis for developing a hypothesis of causation that could be tested by more rigorous observational and experimental studies.

Analytical epidemiology employs carefully selected groups of individuals in an attempt to more convincingly evaluate hypotheses about potential causes for a disease outbreak. The selection of cases is generally made at random, so the results are not biased because of some common characteristic of the study participants. Analytical studies may gather their data by going back in time (retrospective studies), or as events unfold forward in time (prospective studies).

Retrospective studies gather data from the past on present-day cases. Data can include things like the medical history, age, gender, or occupational history of the affected individuals. This type of study examines associations between factors chosen or available to the researcher and disease occurrence.

Prospective studies follow individuals and monitor their disease state during the course of the study. Data on the characteristics of the study subjects and their environments are gathered at the beginning and during

the study so that subjects who become ill may be compared with those who do not. Again, the researchers can look for associations between the disease state and variables that were measured during the study to shed light on possible causes.

Analytical studies incorporate groups into their designs to assist in teasing out associations with disease. Approaches to group-based analytical studies include cohort studies, case-control studies, and cross-sectional studies. The **cohort method** examines groups of individuals (called cohorts) who share a particular characteristic. For example, a cohort might consist of individuals born in the same year and the same place; or it might consist of people who practice or avoid a particular behavior, e.g., smokers or nonsmokers. In a cohort study, cohorts can be followed prospectively or studied retrospectively. If only a single cohort is followed, then the affected individuals are compared with the unaffected individuals in the same group. Disease outcomes are recorded and analyzed to try to identify correlations between characteristics of individuals in the cohort and disease incidence. Cohort studies are a useful way to determine the causes of a condition without violating the ethical prohibition of exposing subjects to a risk factor. Cohorts are typically identified and defined based on suspected risk factors to which individuals have already been exposed through their own choices or circumstances.

Case-control studies are typically retrospective and compare a group of individuals with a disease to a similar group of individuals without the disease. Case-control studies are far more efficient than cohort studies because researchers can deliberately select subjects who are already affected with the disease as opposed to waiting to see which subjects from a random sample will develop a disease.

A **cross-sectional study** analyzes randomly selected individuals in a population and compares individuals affected by a disease or condition to those unaffected at a single point in time. Subjects are compared to look for associations between certain measurable variables and the disease or condition. Cross-sectional studies are also used to determine the prevalence of a condition.

Experimental Studies

Experimental epidemiology uses laboratory or clinical studies in which the investigator manipulates the study subjects to study the connections between diseases and potential causative agents or to assess treatments. Examples of treatments might be the administration of a drug, the inclusion or exclusion of different dietary items, physical exercise, or a particular surgical procedure. Animals or humans are used as test subjects. Because **experimental studies** involve manipulation of subjects, they are typically more difficult and sometimes impossible for ethical reasons.

Koch's postulates require experimental interventions to determine the causative agent for a disease. Unlike observational studies, experimental studies can provide strong evidence supporting cause because other factors are typically held constant when the researcher manipulates the subject. The outcomes for one group receiving the treatment are compared to outcomes for a group that does not receive the treatment but is treated the same in every other way. For example, one group might receive a regimen of a drug administered as a pill, while the untreated group receives a placebo (a pill that looks the same but has no active ingredient). Both groups are treated as similarly as possible except for the administration of the drug. Because other variables are held constant in both the treated and the untreated groups, the researcher is more certain that any change in the treated group is a result of the specific manipulation.

Experimental studies provide the strongest evidence for the etiology of disease, but they must also be designed carefully to eliminate subtle effects of bias. Typically, experimental studies with humans are conducted as double-blind studies, meaning neither the subjects nor the researchers know who is a treatment case and who is not. This design removes a well-known cause of bias in research called the placebo effect, in which knowledge of the treatment by either the subject or the researcher can influence the outcomes.

Note:

- Describe the advantages and disadvantages of observational studies and experimental studies.
- Explain the ways that groups of subjects can be selected for analytical studies.

Note:**Part 3**

Since laboratory tests had confirmed *Salmonella*, a common foodborne pathogen, as the etiologic agent, epidemiologists suspected that the outbreak was caused by contamination at a food processing facility serving the region. Interviews with patients focused on food consumption during and after the Thanksgiving holiday, corresponding with the timing of the outbreak. During the interviews, patients were asked to list items consumed at holiday gatherings and describe how widely each item was consumed among family members and relatives. They were also asked about the sources of food items (e.g., brand, location of purchase, date of purchase). By asking such questions, health officials hoped to identify patterns that would lead back to the source of the outbreak.

Analysis of the interview responses eventually linked almost all of the cases to consumption of a holiday dish known as the turducken—a chicken stuffed inside a duck stuffed inside a turkey. Turducken is a dish not generally consumed year-round, which would explain the spike in cases just after the Thanksgiving holiday. Additional analysis revealed that the turduckens consumed by the affected patients were purchased already stuffed and ready to be cooked. Moreover, the pre-stuffed turduckens were all sold at the same regional grocery chain under two different brand names. Upon further investigation, officials traced both brands to a single processing plant that supplied stores throughout the Florida panhandle.

- Is this an example of common source spread or propagated spread?
- What next steps would the public health office likely take after identifying the source of the outbreak?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Early pioneers of epidemiology such as John Snow, Florence Nightingale, and Joseph Lister, studied disease at the population level and used data to disrupt disease transmission.
- **Descriptive epidemiology** studies rely on case analysis and patient histories to gain information about outbreaks, frequently while they are still occurring.
- **Retrospective epidemiology** studies use historical data to identify associations with the disease state of present cases. **Prospective epidemiology** studies gather data and follow cases to find associations with future disease states.
- **Analytical epidemiology** studies are observational studies that are carefully designed to compare groups and uncover associations between environmental or genetic factors and disease.
- **Experimental epidemiology** studies generate strong evidence of causation in disease or treatment by manipulating subjects and comparing them with control subjects.

Matching

Exercise:

Problem: Match each type of epidemiology study with its description.

____experimental	A. examination of past case histories and medical test results conducted on patients in an outbreak

___analytical	B. examination of current case histories, interviews with patients and their contacts, interpretation of medical test results; frequently conducted while outbreak is still in progress
___prospective	C. use of a set of test subjects (human or animal) and control subjects that are treated the same as the test subjects except for the specific treatment being studied
___descriptive	D. observing groups of individuals to look for associations with disease
___retrospective	E. a comparison of a cohort of individuals through the course of the study

Solution:

C, D, E, B, A

Exercise:

Problem:

Match each pioneer of epidemiology with his or her contribution.

___Florence Nightingale	A. determined the source of a cholera outbreak in London
___Robert Koch	B. showed that surgical wound infection rates could be

	dramatically reduced by using carbolic acid to disinfect surgical tools, bandages, and surgical sites
___Joseph Lister	C. compiled data on causes of mortality in soldiers, leading to innovations in military medical care
___John Snow	D. developed a methodology for conclusively determining the etiology of disease

Solution:

C, D, B, A

Fill in the Blank

Exercise:

Problem:

_____occurs when an infected individual passes the infection on to other individuals, who pass it on to still others, increasing the penetration of the infection into the susceptible population.

Solution:

Propagated spread

Exercise:

Problem:

A batch of food contaminated with botulism exotoxin, consumed at a family reunion by most of the members of a family, would be an example of a _____ outbreak.

Solution:

point source

Short Answer**Exercise:****Problem:**

What activity did John Snow conduct, other than mapping, that contemporary epidemiologists also use when trying to understand how to control a disease?

Modes of Disease Transmission

LEARNING OBJECTIVES

- Describe the different types of disease reservoirs
- Compare contact, vector, and vehicle modes of transmission
- Identify important disease vectors
- Explain the prevalence of nosocomial infections

Understanding how infectious pathogens spread is critical to preventing infectious disease. Many pathogens require a living host to survive, while others may be able to persist in a dormant state outside of a living host. But having infected one host, all pathogens must also have a mechanism of transfer from one host to another or they will die when their host dies. Pathogens often have elaborate adaptations to exploit host biology, behavior, and ecology to live in and move between hosts. Hosts have evolved defenses against pathogens, but because their rates of evolution are typically slower than their pathogens (because their generation times are longer), hosts are usually at an evolutionary disadvantage. This section will explore where pathogens survive—both inside and outside hosts—and some of the many ways they move from one host to another.

Reservoirs and Carriers

For pathogens to persist over long periods of time they require **reservoirs** where they normally reside. Reservoirs can be living organisms or nonliving sites. Nonliving reservoirs can include soil and water in the environment. These may naturally harbor the organism because it may grow in that environment. These environments may also become contaminated

with pathogens in human feces, pathogens shed by intermediate hosts, or pathogens contained in the remains of intermediate hosts.

Pathogens may have mechanisms of dormancy or resilience that allow them to survive (but typically not to reproduce) for varying periods of time in nonliving environments. For example, *Clostridium tetani* survives in the soil and in the presence of oxygen as a resistant endospore. Although many viruses are soon destroyed once in contact with air, water, or other non-physiological conditions, certain types are capable of persisting outside of a living cell for varying amounts of time. For example, a study that looked at the ability of influenza viruses to infect a cell culture after varying amounts of time on a banknote showed survival times from 48 hours to 17 days, depending on how they were deposited on the banknote.[\[footnote\]](#) On the other hand, cold-causing rhinoviruses are somewhat fragile, typically surviving less than a day outside of physiological fluids.

Yves Thomas, Guido Vogel, Werner Wunderli, Patricia Suter, Mark Witschi, Daniel Koch, Caroline Tapparel, and Laurent Kaiser. “Survival of Influenza Virus on Banknotes.” *Applied and Environmental Microbiology* 74, no. 10 (2008): 3002–3007.

A human acting as a reservoir of a pathogen may or may not be capable of transmitting the pathogen, depending on the stage of infection and the pathogen. To help prevent the spread of disease among school children, the CDC has developed guidelines based on the risk of transmission during the course of the disease. For example, children with chickenpox are considered contagious for five days from the start of the rash, whereas children with most gastrointestinal illnesses should be kept home for 24 hours after the symptoms disappear.

An individual capable of transmitting a pathogen without displaying symptoms is referred to as a carrier. A **passive carrier** is contaminated with the pathogen and can mechanically transmit it to another host; however, a passive carrier is not infected. For example, a health-care professional who fails to wash his hands after seeing a patient harboring an infectious agent could become a passive carrier, transmitting the pathogen to another patient who becomes infected.

By contrast, an **active carrier** is an infected individual who can transmit the disease to others. An active carrier may or may not exhibit signs or symptoms of infection. For example, active carriers may transmit the disease during the incubation period (before they show signs and symptoms) or the period of convalescence (after symptoms have subsided). Active carriers who do not present signs or symptoms of disease despite infection are called **asymptomatic carriers**. Pathogens such as hepatitis B virus, herpes simplex virus, and HIV are frequently transmitted by asymptomatic carriers. Mary Mallon, better known as Typhoid Mary, is a famous historical example of an asymptomatic carrier. An Irish immigrant, Mallon worked as a cook for households in and around New York City between 1900 and 1915. In each household, the residents developed typhoid fever (caused by *Salmonella typhi*) a few weeks after Mallon started working. Later investigations determined that Mallon was responsible for at least 122 cases of typhoid fever, five of which were fatal.[\[footnote\]](#) See [Eye on Ethics: Typhoid Mary](#) for more about the Mallon case.

Filio Marineli, Gregory Tsoucalas, Marianna Karamanou, and George Androustos. “Mary Mallon (1869–1938) and the History of Typhoid Fever.” *Annals of Gastroenterology* 26 (2013): 132–134.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3959940/pdf/AnnGastroenterol-26-132.pdf>.

A pathogen may have more than one living reservoir. In zoonotic diseases, animals act as reservoirs of human disease and transmit the infectious agent to humans through direct or indirect contact. In some cases, the disease also affects the animal, but in other cases the animal is asymptomatic.

In parasitic infections, the parasite’s preferred host is called the **definitive host**. In parasites with complex life cycles, the definitive host is the host in which the parasite reaches sexual maturity. Some parasites may also infect one or more **intermediate hosts** in which the parasite goes through several immature life cycle stages or reproduces asexually.

Note:

George Soper, the sanitary engineer who traced the typhoid outbreak to Mary Mallon, [gives an account](#) of his investigation, an example of

descriptive epidemiology, in “The Curious Career of Typhoid Mary.”

Note:

- List some nonliving reservoirs for pathogens.
- Explain the difference between a passive carrier and an active carrier.

Transmission

Regardless of the reservoir, transmission must occur for an infection to spread. First, transmission from the reservoir to the individual must occur. Then, the individual must transmit the infectious agent to other susceptible individuals, either directly or indirectly. Pathogenic microorganisms employ diverse transmission mechanisms.

Contact Transmission

Contact transmission includes direct contact or indirect contact. Person-to-person transmission is a form of **direct contact transmission**. Here the agent is transmitted by physical contact between two individuals ([\[link\]](#)) through actions such as touching, kissing, sexual intercourse, or droplet sprays. Direct contact can be categorized as vertical, horizontal, or droplet transmission. **Vertical direct contact transmission** occurs when pathogens are transmitted from mother to child during pregnancy, birth, or breastfeeding. Other kinds of direct contact transmission are called **horizontal direct contact transmission**. Often, contact between mucous membranes is required for entry of the pathogen into the new host, although skin-to-skin contact can lead to mucous membrane contact if the new host subsequently touches a mucous membrane. Contact transmission may also be site-specific; for example, some diseases can be transmitted by sexual contact but not by other forms of contact.

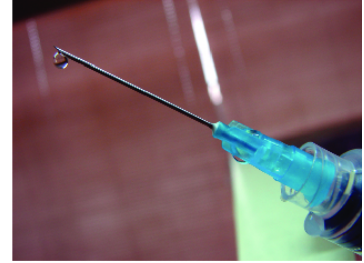
When an individual coughs or sneezes, small droplets of mucus that may contain pathogens are ejected. This leads to direct **droplet transmission**, which refers to droplet transmission of a pathogen to a new host over distances of one meter or less. A wide variety of diseases are transmitted by droplets, including influenza and many forms of pneumonia. Transmission over distances greater than one meter is called airborne transmission.

Indirect contact transmission involves inanimate objects called fomites that become contaminated by pathogens from an infected individual or reservoir ([\[link\]](#)). For example, an individual with the common cold may sneeze, causing droplets to land on a fomite such as a tablecloth or carpet, or the individual may wipe her nose and then transfer mucus to a fomite such as a doorknob or towel. Transmission occurs indirectly when a new susceptible host later touches the fomite and transfers the contaminated material to a susceptible portal of entry. Fomites can also include objects used in clinical settings that are not properly sterilized, such as syringes, needles, catheters, and surgical equipment. Pathogens transmitted indirectly via such fomites are a major cause of healthcare-associated infections (see [Controlling Microbial Growth](#)).



Direct contact transmission of pathogens can occur through physical contact. Many pathogens require contact with a mucous membrane to enter the body, but the host may transfer the pathogen from another

point of contact (e.g., hand) to a mucous membrane (e.g., mouth or eye). (credit left: modification of work by Lisa Doehnert)



Fomites are nonliving objects that facilitate the indirect transmission of pathogens. Contaminated doorknobs, towels, and syringes are all common examples of fomites. (credit left: modification of work by Kate Ter Haar; credit middle: modification of work by Vernon Swanepoel; credit right: modification of work by “Zaldylmg”/Flickr)

Vehicle Transmission

The term **vehicle transmission** refers to the transmission of pathogens through vehicles such as water, food, and air. Water contamination through poor sanitation methods leads to waterborne transmission of disease. Waterborne disease remains a serious problem in many regions throughout the world. The World Health Organization (WHO) estimates that contaminated drinking water is responsible for more than 500,000 deaths each year.[\[footnote\]](#) Similarly, food contaminated through poor handling or storage can lead to foodborne transmission of disease ([\[link\]](#)).

World Health Organization. Fact sheet No. 391—*Drinking Water*. June 2005. <http://www.who.int/mediacentre/factsheets/fs391/en>.

Dust and fine particles known as aerosols, which can float in the air, can carry pathogens and facilitate the airborne transmission of disease. For

example, dust particles are the dominant mode of transmission of hantavirus to humans. Hantavirus is found in mouse feces, urine, and saliva, but when these substances dry, they can disintegrate into fine particles that can become airborne when disturbed; inhalation of these particles can lead to a serious and sometimes fatal respiratory infection.

Although droplet transmission over short distances is considered contact transmission as discussed above, longer distance transmission of droplets through the air is considered vehicle transmission. Unlike larger particles that drop quickly out of the air column, fine mucus droplets produced by coughs or sneezes can remain suspended for long periods of time, traveling considerable distances. In certain conditions, droplets desiccate quickly to produce a droplet nucleus that is capable of transmitting pathogens; air temperature and humidity can have an impact on effectiveness of airborne transmission.

Tuberculosis is often transmitted via airborne transmission when the causative agent, *Mycobacterium tuberculosis*, is released in small particles with coughs. Because tuberculosis requires as few as 10 microbes to initiate a new infection, patients with tuberculosis must be treated in rooms equipped with special ventilation, and anyone entering the room should wear a mask.



Food is an important vehicle of transmission for pathogens, especially of the gastrointestinal and upper respiratory systems. Notice the glass shield above the food trays, designed to prevent pathogens ejected in coughs and sneezes from entering the food. (credit: Fort George G. Meade Public Affairs Office)

Note:**Resolution**

After identifying the source of the contaminated turduckens, the Florida public health office notified the CDC, which requested an expedited inspection of the facility by state inspectors. Inspectors found that a machine used to process the chicken was contaminated with *Salmonella* as a result of substandard cleaning protocols. Inspectors also found that the process of stuffing and packaging the turduckens prior to refrigeration allowed the meat to remain at temperatures conducive to bacterial growth

for too long. The contamination and the delayed refrigeration led to vehicle (food) transmission of the bacteria in turduckens.

Based on these findings, the plant was shut down for a full and thorough decontamination. All turduckens produced in the plant were recalled and pulled from store shelves ahead of the December holiday season, preventing further outbreaks.

Go back to the [previous](#) Clinical Focus Box.

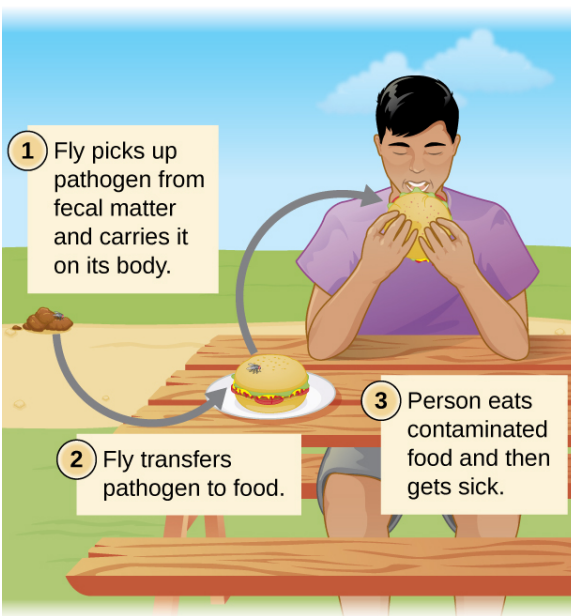
Vector Transmission

Diseases can also be transmitted by a mechanical or biological vector, an animal (typically an arthropod) that carries the disease from one host to another. **Mechanical transmission** is facilitated by a **mechanical vector**, an animal that carries a pathogen from one host to another without being infected itself. For example, a fly may land on fecal matter and later transmit bacteria from the feces to food that it lands on; a human eating the food may then become infected by the bacteria, resulting in a case of diarrhea or dysentery ([link](#)).

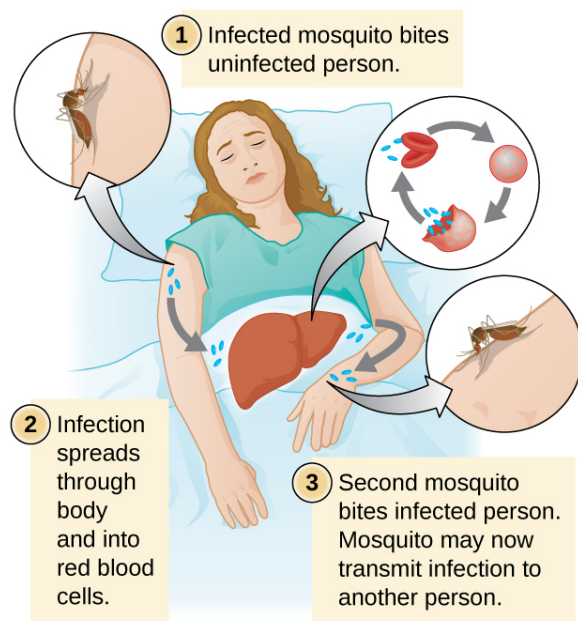
Biological transmission occurs when the pathogen reproduces within a **biological vector** that transmits the pathogen from one host to another ([link](#)). Arthropods are the main vectors responsible for biological transmission ([link](#)). Most arthropod vectors transmit the pathogen by biting the host, creating a wound that serves as a portal of entry. The pathogen may go through part of its reproductive cycle in the gut or salivary glands of the arthropod to facilitate its transmission through the bite. For example, hemipterans (called “kissing bugs” or “assassin bugs”) transmit Chagas disease to humans by defecating when they bite, after which the human scratches or rubs the infected feces into a mucous membrane or break in the skin.

Biological insect vectors include mosquitoes, which transmit malaria and other diseases, and lice, which transmit typhus. Other arthropod vectors can include arachnids, primarily ticks, which transmit Lyme disease and other diseases, and mites, which transmit scrub typhus and rickettsial pox. Biological transmission, because it involves survival and reproduction

within a parasitized vector, complicates the biology of the pathogen and its transmission. There are also important non-arthropod vectors of disease, including mammals and birds. Various species of mammals can transmit rabies to humans, usually by means of a bite that transmits the rabies virus. Chickens and other domestic poultry can transmit avian influenza to humans through direct or indirect contact with avian influenza virus. A shed in the birds' saliva, mucous, and feces.

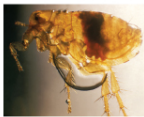


(a)



(b)

(a) A mechanical vector carries a pathogen on its body from one host to another, not as an infection. (b) A biological vector carries a pathogen from one host to another after becoming infected itself.

Common Arthropod Vectors and Select Pathogens			
Vector	Species	Pathogen	Disease
Black fly 	<i>Simulium</i> spp.	<i>Onchocerca volvulus</i>	Onchocerciasis (river blindness)
Flea 	<i>Xenopsylla cheopis</i>	<i>Rickettsia typhi</i>	Murine typhus
		<i>Yersinia pestis</i>	Plague
Kissing bug 	<i>Triatoma</i> spp.	<i>Trypanosoma cruzi</i>	Chagas disease
Louse 	<i>Pediculus humanus humanus</i>	<i>Bartonella quintana</i>	Trench fever
		<i>Borrelia recurrentis</i>	Relapsing fever
		<i>Rickettsia prowazekii</i>	Typhus
Mite (chigger) 	<i>Leptotrombidium</i> spp.	<i>Orientia tsutsugamushi</i>	Scrub typhus
	<i>Liponyssoides sanguineus</i>	<i>Rickettsia akari</i>	Rickettsialpox
Mosquito 	<i>Aedes</i> spp., <i>Haemagogus</i> spp.	<i>Yellow fever virus</i>	Yellow fever
	<i>Anopheles</i> spp.	<i>Plasmodium falciparum</i>	Malaria
	<i>Culex pipiens</i>	<i>West Nile virus</i>	West Nile disease
Sand fly 	<i>Phlebotomus</i> spp.	<i>Leishmania</i> spp.	Leishmaniasis
Tick 	<i>Ixodes</i> spp.	<i>Borrelia</i> spp.	Lyme disease
	<i>Dermacentor</i> spp. and others	<i>Rickettsia rickettsii</i>	Rocky Mountain spotted fever
Tsetse fly 	<i>Glossina</i> spp.	<i>Trypanosoma brucei</i>	African trypanosomiasis (sleeping sickness)

(credit “Black fly”, “Tick”, “Tsetse fly”: modification of work by USDA; credit: “Flea”: modification of work by Centers for Disease Control and Prevention; credit: “Louse”, “Mosquito”, “Sand fly”:

modification of work by James Gathany, Centers for Disease Control and Prevention; credit “Kissing bug”: modification of work by Glenn Seplak; credit “Mite”: modification of work by Michael Wunderli)

Note:

- Describe how diseases can be transmitted through the air.
- Explain the difference between a mechanical vector and a biological vector.

Note:

Using GMOs to Stop the Spread of Zika

In 2016, an epidemic of the Zika virus was linked to a high incidence of birth defects in South America and Central America. As winter turned to spring in the northern hemisphere, health officials correctly predicted the virus would spread to North America, coinciding with the breeding season of its major vector, the *Aedes aegypti* mosquito.

The range of the *A. aegypti* mosquito extends well into the southern United States ([link](#)). Because these same mosquitoes serve as vectors for other problematic diseases (dengue fever, yellow fever, and others), various methods of mosquito control have been proposed as solutions. Chemical pesticides have been used effectively in the past, and are likely to be used again; but because chemical pesticides can have negative impacts on the environment, some scientists have proposed an alternative that involves genetically engineering *A. aegypti* so that it cannot reproduce. This method, however, has been the subject of some controversy.

One method that has worked in the past to control pests, with little apparent downside, has been sterile male introductions. This method controlled the screw-worm fly pest in the southwest United States and fruit fly pests of fruit crops. In this method, males of the target species are reared in the lab, sterilized with radiation, and released into the

environment where they mate with wild females, who subsequently bear no live offspring. Repeated releases shrink the pest population.

A similar method, taking advantage of recombinant DNA technology, [\[footnote\]](#) introduces a dominant lethal allele into male mosquitoes that is suppressed in the presence of tetracycline (an antibiotic) during laboratory rearing. The males are released into the environment and mate with female mosquitoes. Unlike the sterile male method, these matings produce offspring, but they die as larvae from the lethal gene in the absence of tetracycline in the environment. As of 2016, this method has yet to be implemented in the United States, but a UK company tested the method in Piracicaba, Brazil, and found an 82% reduction in wild *A. aegypti* larvae and a 91% reduction in dengue cases in the treated area. [\[footnote\]](#) In August 2016, amid news of Zika infections in several Florida communities, the FDA gave the UK company permission to test this same mosquito control method in Key West, Florida, pending compliance with local and state regulations and a referendum in the affected communities.

Blandine Massonnet-Bruneel, Nicole Corre-Catelin, Renaud Lacroix, Rosemary S. Lees, Kim Phuc Hoang, Derric Nimmo, Luke Alphey, and Paul Reiter. “Fitness of Transgenic Mosquito *Aedes aegypti* Males Carrying a Dominant Lethal Genetic System.” *PLOS ONE* 8, no. 5 (2013): e62711.

Richard Levine. “Cases of Dengue Drop 91 Percent Due to Genetically Modified Mosquitoes.” *Entomology Today*.

<https://entomologytoday.org/2016/07/14/cases-of-dengue-drop-91-due-to-genetically-modified-mosquitoes>.

The use of genetically modified organisms (GMOs) to control a disease vector has its advocates as well as its opponents. In theory, the system could be used to drive the *A. aegypti* mosquito extinct—a noble goal according to some, given the damage they do to human populations.

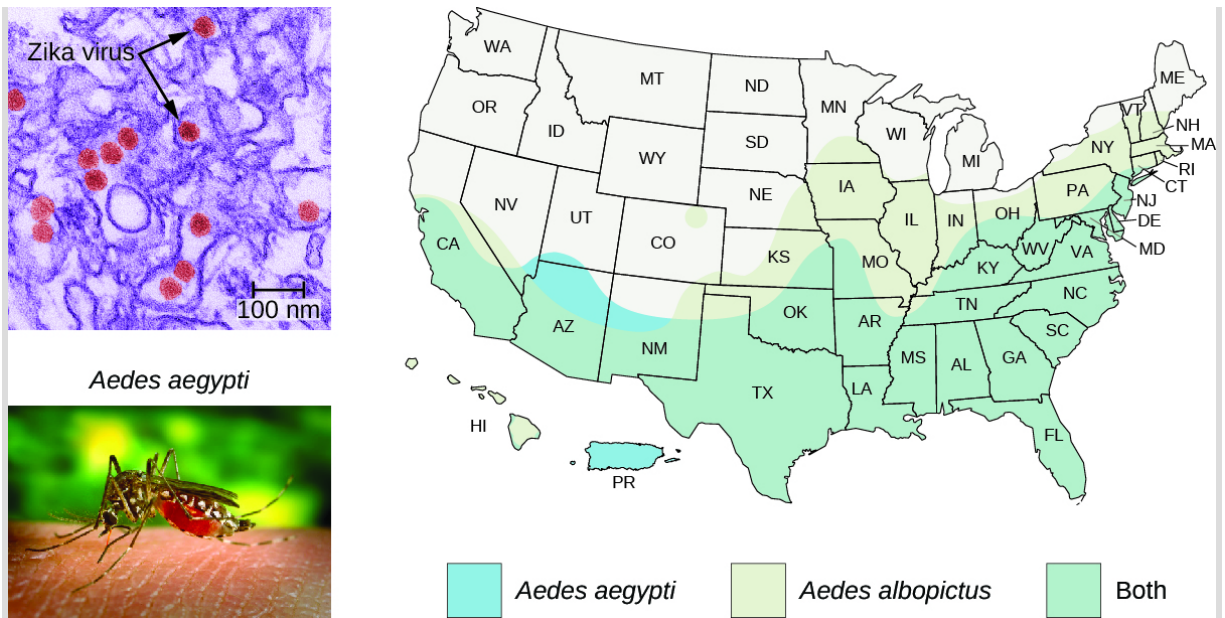
[\[footnote\]](#) But opponents of the idea are concerned that the gene could escape the species boundary of *A. aegypti* and cause problems in other species, leading to unforeseen ecological consequences. Opponents are also wary of the program because it is being administered by a for-profit corporation, creating the potential for conflicts of interest that would have to be tightly regulated; and it is not clear how any unintended consequences of the program could be reversed.

Olivia Judson. “A Bug’s Death.” *The New York Times*, September 25, 2003. <http://www.nytimes.com/2003/09/25/opinion/a-bug-s-death.html>. There are other epidemiological considerations as well. *Aedes aegypti* is apparently not the only vector for the Zika virus. *Aedes albopictus*, the Asian tiger mosquito, is also a vector for the Zika virus.^[footnote] *A. albopictus* is now widespread around the planet including much of the United States ([\[link\]](#)). Many other mosquitoes have been found to harbor Zika virus, though their capacity to act as vectors is unknown.^[footnote] Genetically modified strains of *A. aegypti* will not control the other species of vectors. Finally, the Zika virus can apparently be transmitted sexually between human hosts, from mother to child, and possibly through blood transfusion. All of these factors must be considered in any approach to controlling the spread of the virus.

Gilda Grard, Mélanie Caron, Illich Manfred Mombo, Dieudonné Nkoghe, Statiana Mbouï Ondo, Davy Jiolle, Didier Fontenille, Christophe Paupy, and Eric Maurice Leroy. “Zika Virus in Gabon (Central Africa)–2007: A New Threat from *Aedes albopictus*?” *PLOS Neglected Tropical Diseases* 8, no. 2 (2014): e2681.

Constância F.J. Ayres. “Identification of Zika Virus Vectors and Implications for Control.” *The Lancet Infectious Diseases* 16, no. 3 (2016): 278–279.

Clearly there are risks and unknowns involved in conducting an open-environment experiment of an as-yet poorly understood technology. But allowing the Zika virus to spread unchecked is also risky. Does the threat of a Zika epidemic justify the ecological risk of genetically engineering mosquitos? Are current methods of mosquito control sufficiently ineffective or harmful that we need to try untested alternatives? These are the questions being put to public health officials now.



The Zika virus is an enveloped virus transmitted by mosquitoes, especially *Aedes aegypti*. The range of this mosquito includes much of the United States, from the Southwest and Southeast to as far north as the Mid-Atlantic. The range of *A. albopictus*, another vector, extends even farther north to New England and parts of the Midwest. (credit micrograph: modification of work by Cynthia Goldsmith, Centers for Disease Control and Prevention; credit photo: modification of work by James Gathany, Centers for Disease Control and Prevention; credit map: modification of work by Centers for Disease Control and Prevention)

Quarantining

Individuals suspected or known to have been exposed to certain contagious pathogens may be **quarantined**, or isolated to prevent transmission of the disease to others. Hospitals and other health-care facilities generally set up special wards to isolate patients with particularly hazardous diseases such as tuberculosis or Ebola ([link](#)). Depending on the setting, these wards may be equipped with special air-handling methods, and personnel may

implement special protocols to limit the risk of transmission, such as personal protective equipment or the use of chemical disinfectant sprays upon entry and exit of medical personnel.

The duration of the quarantine depends on factors such as the incubation period of the disease and the evidence suggestive of an infection. The patient may be released if signs and symptoms fail to materialize when expected or if preventive treatment can be administered in order to limit the risk of transmission. If the infection is confirmed, the patient may be compelled to remain in isolation until the disease is no longer considered contagious.

In the United States, public health authorities may only quarantine patients for certain diseases, such as cholera, diphtheria, infectious tuberculosis, and strains of influenza capable of causing a pandemic. Individuals entering the United States or moving between states may be quarantined by the CDC if they are suspected of having been exposed to one of these diseases. Although the CDC routinely monitors entry points to the United States for crew or passengers displaying illness, quarantine is rarely implemented.



(a)



(b)

(a) The Aeromedical Biological Containment System (ABCS) is a module designed by the CDC and Department of Defense specifically for transporting highly contagious patients by air. (b) An isolation ward for Ebola patients in Lagos, Nigeria. (credit a: modification of

work by Centers for Disease Control and Prevention; credit b:
modification of work by CDC Global)

Healthcare-Associated (Nosocomial) Infections

Hospitals, retirement homes, and prisons attract the attention of epidemiologists because these settings are associated with increased incidence of certain diseases. Higher rates of transmission may be caused by characteristics of the environment itself, characteristics of the population, or both. Consequently, special efforts must be taken to limit the risks of infection in these settings.

Infections acquired in health-care facilities, including hospitals, are called **nosocomial infections** or **healthcare-associated infections (HAI)**. HAIs are often connected with surgery or other invasive procedures that provide the pathogen with access to the portal of infection. For an infection to be classified as an HAI, the patient must have been admitted to the health-care facility for a reason other than the infection. In these settings, patients suffering from primary disease are often afflicted with compromised immunity and are more susceptible to secondary infection and opportunistic pathogens.

In 2011, more than 720,000 HAIs occurred in hospitals in the United States, according to the CDC. About 22% of these HAIs occurred at a surgical site, and cases of pneumonia accounted for another 22%; urinary tract infections accounted for an additional 13%, and primary bloodstream infections 10%. [\[footnote\]](#) Such HAIs often occur when pathogens are introduced to patients' bodies through contaminated surgical or medical equipment, such as catheters and respiratory ventilators. Health-care facilities seek to limit nosocomial infections through training and hygiene protocols such as those described in [Control of Microbial Growth](#).

Centers for Disease Control and Prevention. "HAI Data and Statistics." 2016. <http://www.cdc.gov/hai/surveillance>. Accessed Jan 2, 2016.

Note:

- Give some reasons why HAIs occur.

Key Concepts and Summary

- **Reservoirs** of human disease can include the human and animal populations, soil, water, and inanimate objects or materials.
- **Contact transmission** can be **direct** or **indirect** through physical contact with either an infected host (direct) or contact with a fomite that an infected host has made contact with previously (indirect).
- Vector transmission occurs when a living organism carries an infectious agent on its body (**mechanical**) or as an infection host itself (**biological**), to a new host.
- **Vehicle transmission** occurs when a substance, such as soil, water, or air, carries an infectious agent to a new host.
- **Healthcare-associated infections (HAI)**, or **nosocomial infections**, are acquired in a clinical setting. Transmission is facilitated by medical interventions and the high concentration of susceptible, immunocompromised individuals in clinical settings.

Multiple Choice

Exercise:**Problem:**

Which is the most common type of biological vector of human disease?

- a. viruses
 - b. bacteria
 - c. mammals
 - d. arthropods
-

Solution:

D

Exercise:**Problem:**

A mosquito bites a person who subsequently develops a fever and abdominal rash. What type of transmission would this be?

- a. mechanical vector transmission
- b. biological vector transmission
- c. direct contact transmission
- d. vehicle transmission

Solution:

B

Exercise:**Problem:**

Cattle are allowed to pasture in a field that contains the farmhouse well, and the farmer's family becomes ill with a gastrointestinal pathogen after drinking the water. What type of transmission of infectious agents would this be?

- a. biological vector transmission
- b. direct contact transmission
- c. indirect contact transmission
- d. vehicle transmission

Solution:

D

Exercise:

Problem:

A blanket from a child with chickenpox is likely to be contaminated with the virus that causes chickenpox (Varicella-zoster virus). What is the blanket called?

- a. fomite
- b. host
- c. pathogen
- d. vector

Solution:

A

Fill in the Blank**Exercise:****Problem:**

A patient in the hospital with a urinary catheter develops a bladder infection. This is an example of a(n) _____ infection.

Solution:

nosocomial or healthcare-associated

Exercise:**Problem:**

A _____ is an animal that can transfer infectious pathogens from one host to another.

Solution:

vector

Short Answer

Exercise:

Problem:

Differentiate between droplet vehicle transmission and airborne transmission.

Critical Thinking

Exercise:

Problem:

Many people find that they become ill with a cold after traveling by airplane. The air circulation systems of commercial aircraft use HEPA filters that should remove any infectious agents that pass through them. What are the possible reasons for increased incidence of colds after flights?

Introduction

class="introduction"

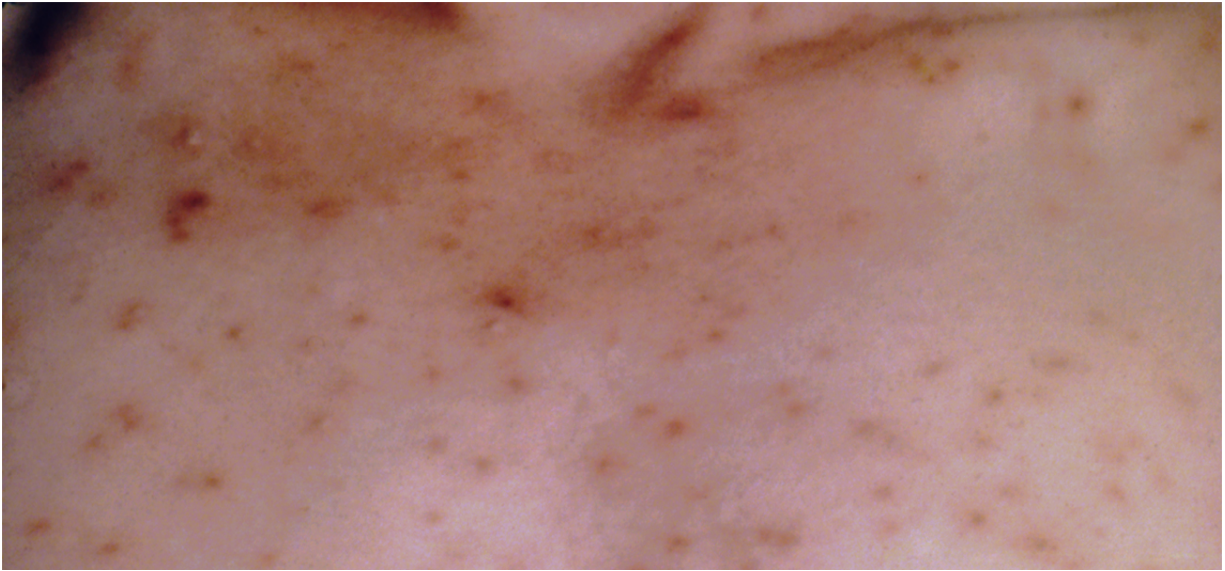
Varicella, or chickenpox, is caused by the highly contagious varicella-zoster virus.

The characteristic rash seen here is partly a result of inflammation associated with the body's immune response to the virus.

Inflammation is a response mechanism of innate immunity that helps the body fight off a wide range of infections.

(credit: modification of work by Centers for

Disease Control and Prevention)



Despite relatively constant exposure to pathogenic microbes in the environment, humans do not generally suffer from constant infection or disease. Under most circumstances, the body is able to defend itself from the threat of infection thanks to a complex immune system designed to repel, kill, and expel disease-causing invaders. Immunity as a whole can be described as two interrelated parts: nonspecific innate immunity, which is the subject of this chapter, and specific adaptive host defenses, which are discussed in the next chapter.

The nonspecific innate immune response provides a first line of defense that can often prevent infections from gaining a solid foothold in the body. These defenses are described as *nonspecific* because they do not target any specific pathogen; rather, they defend against a wide range of potential pathogens. They are called *innate* because they are built-in mechanisms of the human organism. Unlike the specific adaptive defenses, they are not acquired over time and they have no “memory” (they do not improve after repeated exposures to specific pathogens).

Broadly speaking, nonspecific innate defenses provide an immediate (or very rapid) response against potential pathogens. However, these responses are neither perfect nor impenetrable. They can be circumvented by pathogens on occasion, and sometimes they can even cause damage to the body, contributing to the signs and symptoms of infection ([\[link\]](#)).

Physical Defenses

LEARNING OBJECTIVES

- Describe the various physical barriers and mechanical defenses that protect the human body against infection and disease
- Describe the role of microbiota as a first-line defense against infection and disease

Note:

Part 1

Angela, a 25-year-old female patient in the emergency department, is having some trouble communicating verbally because of shortness of breath. A nurse observes constriction and swelling of the airway and labored breathing. The nurse asks Angela if she has a history of asthma or allergies. Angela shakes her head no, but there is fear in her eyes. With some difficulty, she explains that her father died suddenly at age 27, when she was just a little girl, of a similar respiratory attack. The underlying cause had never been identified.

- What are some possible causes of constriction and swelling of the airway?
- What causes swelling of body tissues in general?

Jump to the [next](#) Clinical Focus box.

Nonspecific innate immunity can be characterized as a multifaceted system of defenses that targets invading pathogens in a nonspecific manner. In this chapter, we have divided the numerous defenses that make up this system into three categories: physical defenses, chemical defenses, and cellular defenses. However, it is important to keep in mind that these defenses do not function independently, and the categories often overlap. [\[link\]](#) provides an overview of the nonspecific defenses discussed in this chapter.

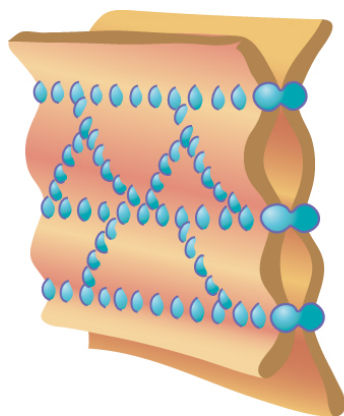
Overview of Nonspecific Innate Immune Defenses	
Physical defenses	Physical barriers
	Mechanical defenses
	Microbiome
Chemical defenses	Chemicals and enzymes in body fluids
	Antimicrobial peptides
	Plasma protein mediators
	Cytokines
	Inflammation-eliciting mediators
Cellular defenses	Granulocytes
	Agranulocytes

Physical defenses provide the body’s most basic form of nonspecific defense. They include physical barriers to microbes, such as the skin and

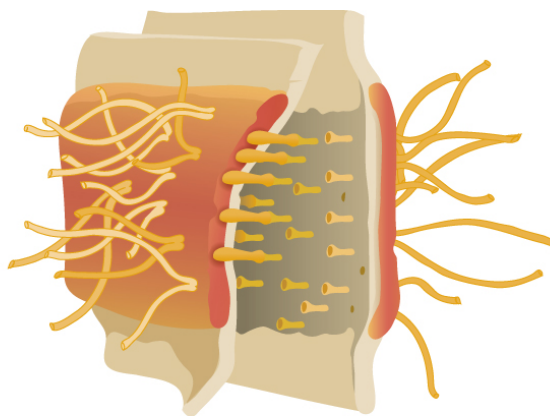
mucous membranes, as well as mechanical defenses that physically remove microbes and debris from areas of the body where they might cause harm or infection. In addition, the microbiome provides a measure of physical protection against disease, as microbes of the normal microbiota compete with pathogens for nutrients and cellular binding sites necessary to cause infection.

Physical Barriers

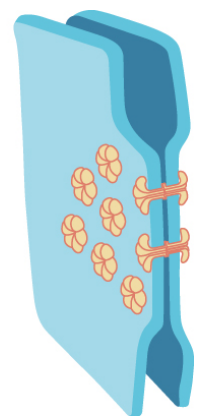
Physical barriers play an important role in preventing microbes from reaching tissues that are susceptible to infection. At the cellular level, barriers consist of cells that are tightly joined to prevent invaders from crossing through to deeper tissue. For example, the endothelial cells that line blood vessels have very tight cell-to-cell junctions, blocking microbes from gaining access to the bloodstream. Cell junctions are generally composed of cell membrane proteins that may connect with the extracellular matrix or with complementary proteins from neighboring cells. Tissues in various parts of the body have different types of cell junctions. These include tight junctions, desmosomes, and gap junctions, as illustrated in [\[link\]](#). Invading microorganisms may attempt to break down these substances chemically, using enzymes such as proteases that can cause structural damage to create a point of entry for pathogens.



tight junctions



desmosomes



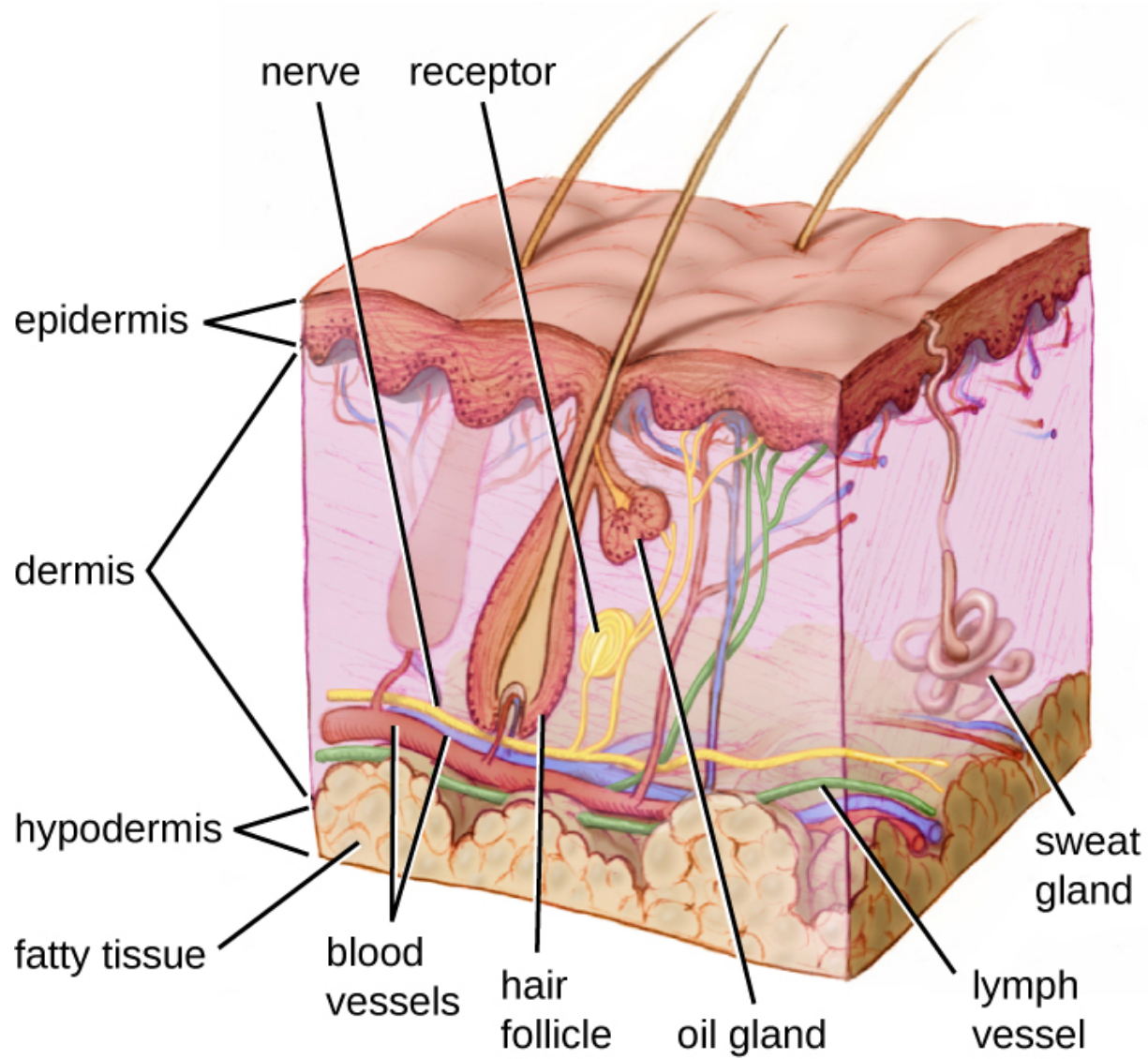
gap junctions

There are multiple types of cell junctions in human tissue, three of

which are shown here. Tight junctions rivet two adjacent cells together, preventing or limiting material exchange through the spaces between them. Desmosomes have intermediate fibers that act like shoelaces, tying two cells together, allowing small materials to pass through the resulting spaces. Gap junctions are channels between two cells that permit their communication via signals. (credit: modification of work by Mariana Ruiz Villareal)

The Skin Barrier

One of the body's most important physical barriers is the skin barrier, which is composed of three layers of closely packed cells. The thin upper layer is called the epidermis. A second, thicker layer, called the dermis, contains hair follicles, sweat glands, nerves, and blood vessels. A layer of fatty tissue called the hypodermis lies beneath the dermis and contains blood and lymph vessels ([link](#)).



Human skin has three layers, the epidermis, the dermis, and the hypodermis, which provide a thick barrier between microbes outside the body and deeper tissues. Dead skin cells on the surface of the epidermis are continually shed, taking with them microbes on the skin's surface. (credit: modification of work by National Institutes of Health)

The topmost layer of skin, the epidermis, consists of cells that are packed with keratin. These dead cells remain as a tightly connected, dense layer of

protein-filled cell husks on the surface of the skin. The keratin makes the skin's surface mechanically tough and resistant to degradation by bacterial enzymes. Fatty acids on the skin's surface create a dry, salty, and acidic environment that inhibits the growth of some microbes and is highly resistant to breakdown by bacterial enzymes. In addition, the dead cells of the epidermis are frequently shed, along with any microbes that may be clinging to them. Shed skin cells are continually replaced with new cells from below, providing a new barrier that will soon be shed in the same way.

Infections can occur when the skin barrier is compromised or broken. A wound can serve as a point of entry for opportunistic pathogens, which can infect the skin tissue surrounding the wound and possibly spread to deeper tissues.

Note:**Every Rose Has its Thorn**

Mike, a gardener from southern California, recently noticed a small red bump on his left forearm. Initially, he did not think much of it, but soon it grew larger and then ulcerated (opened up), becoming a painful lesion that extended across a large part of his forearm ([\[link\]](#)). He went to an urgent care facility, where a physician asked about his occupation. When he said he was a landscaper, the physician immediately suspected a case of sporotrichosis, a type of fungal infection known as rose gardener's disease because it often afflicts landscapers and gardening enthusiasts.

Under most conditions, fungi cannot produce skin infections in healthy individuals. Fungi grow filaments known as hyphae, which are not particularly invasive and can be easily kept at bay by the physical barriers of the skin and mucous membranes. However, small wounds in the skin, such as those caused by thorns, can provide an opening for opportunistic pathogens like *Sporothrix schenckii*, a soil-dwelling fungus and the causative agent of rose gardener's disease. Once it breaches the skin barrier, *S. schenckii* can infect the skin and underlying tissues, producing ulcerated lesions like Mike's. Compounding matters, other pathogens may enter the infected tissue, causing secondary bacterial infections.

Luckily, rose gardener's disease is treatable. Mike's physician wrote him a prescription for some antifungal drugs as well as a course of antibiotics to combat secondary bacterial infections. His lesions eventually healed, and Mike returned to work with a new appreciation for gloves and protective clothing.



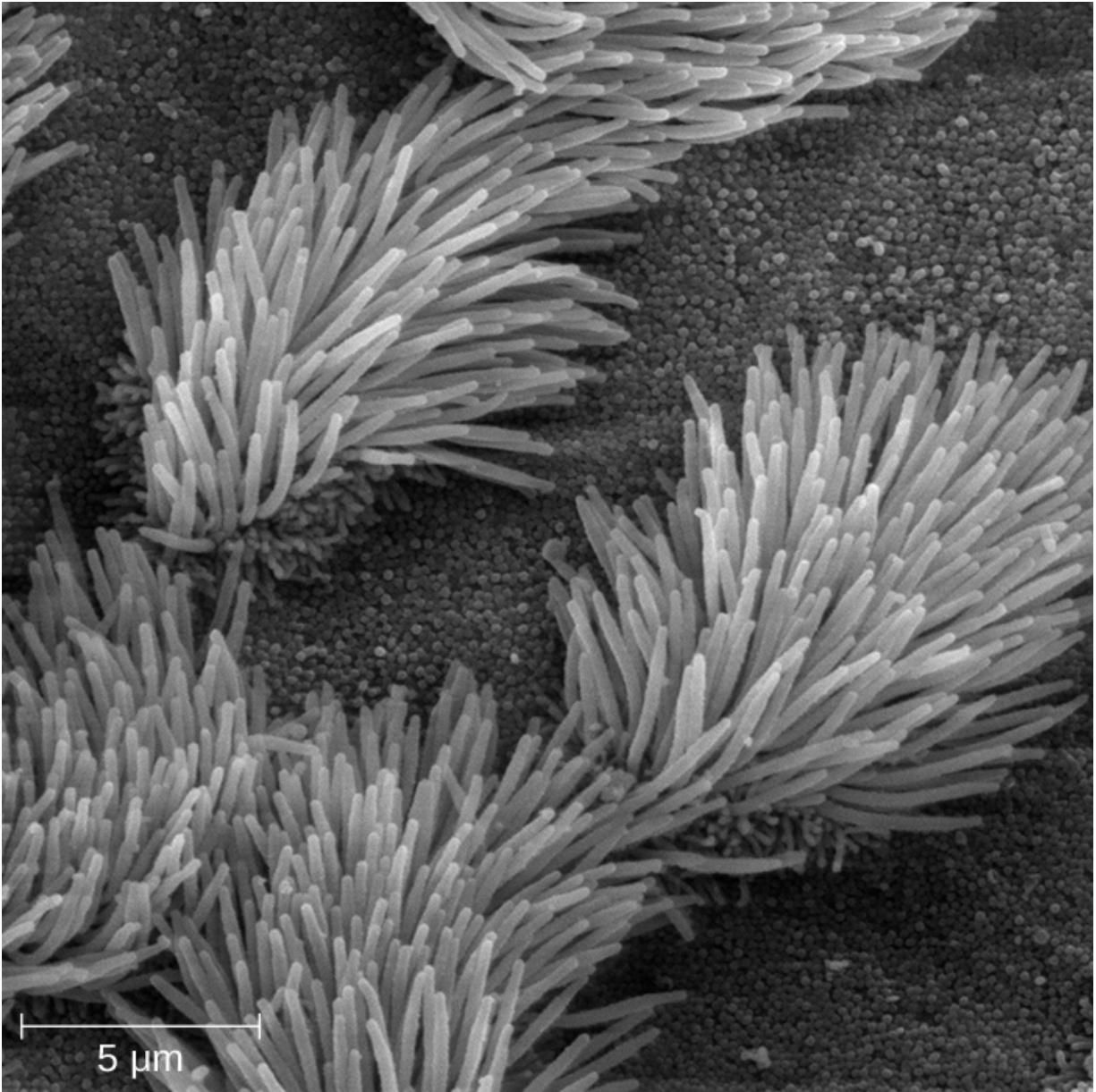
Rose gardener's disease can occur when the fungus *Sporothrix schenckii* breaches the skin through small cuts, such as might be inflicted by thorns. (credit left: modification of work by Elisa Self; credit right: modification of work by Centers for Disease Control and Prevention)

Mucous Membranes

The **mucous membranes** lining the nose, mouth, lungs, and urinary and digestive tracts provide another nonspecific barrier against potential pathogens. Mucous membranes consist of a layer of epithelial cells bound by tight junctions. The epithelial cells secrete a moist, sticky substance called **mucus**, which covers and protects the more fragile cell layers beneath it and traps debris and particulate matter, including microbes. Mucus secretions also contain antimicrobial peptides.

In many regions of the body, mechanical actions serve to flush mucus (along with trapped or dead microbes) out of the body or away from potential sites of infection. For example, in the respiratory system,

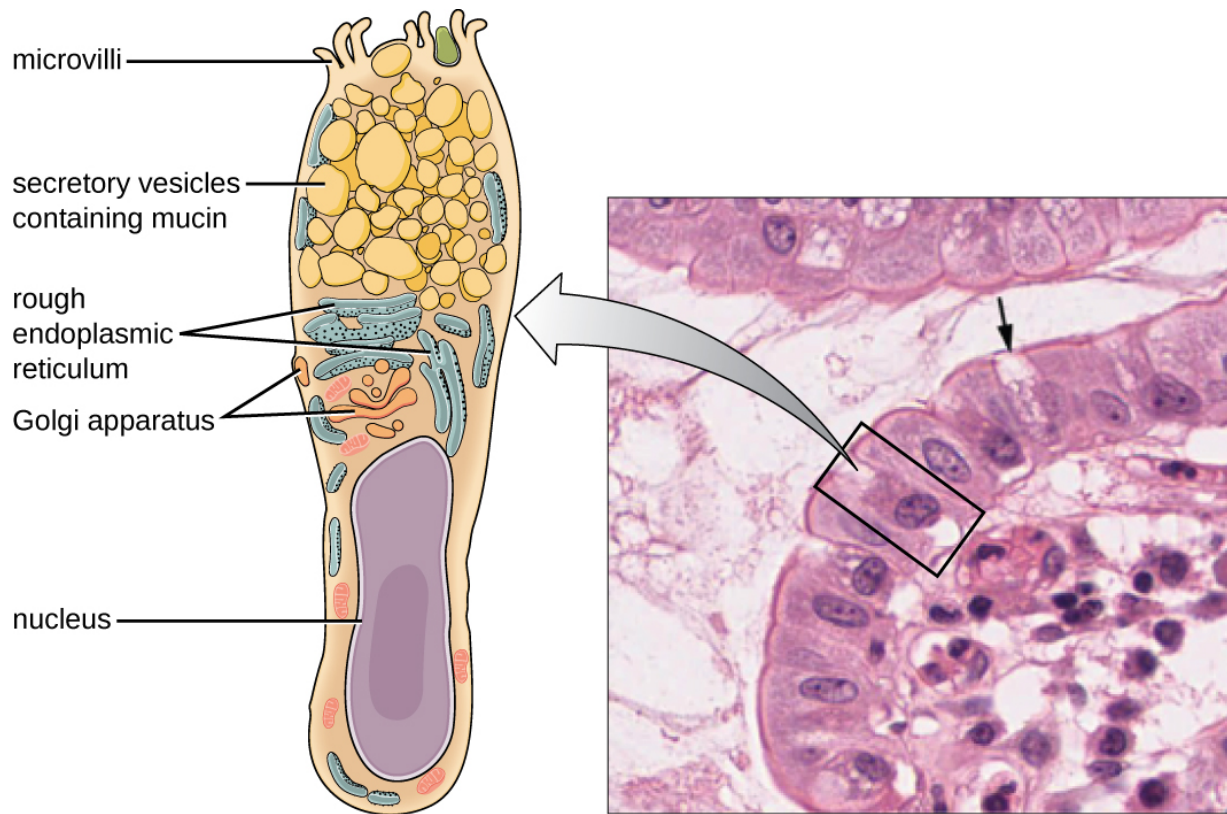
inhalation can bring microbes, dust, mold spores, and other small airborne debris into the body. This debris becomes trapped in the mucus lining the respiratory tract, a layer known as the mucociliary blanket. The epithelial cells lining the upper parts of the respiratory tract are called **ciliated epithelial cells** because they have hair-like appendages known as cilia. Movement of the cilia propels debris-laden mucus out and away from the lungs. The expelled mucus is then swallowed and destroyed in the stomach, or coughed up, or sneezed out ([\[link\]](#)). This system of removal is often called the **mucociliary escalator**.



This scanning electron micrograph shows ciliated and nonciliated epithelial cells from the human trachea. The mucociliary escalator pushes mucus away from the lungs, along with any debris or microorganisms that may be trapped in the sticky mucus, and the mucus moves up to the esophagus where it can be removed by swallowing.

The mucociliary escalator is such an effective barrier to microbes that the lungs, the lowermost (and most sensitive) portion of the respiratory tract, were long considered to be a sterile environment in healthy individuals. Only recently has research suggested that healthy lungs may have a small normal microbiota. Disruption of the mucociliary escalator by the damaging effects of smoking or diseases such as cystic fibrosis can lead to increased colonization of bacteria in the lower respiratory tract and frequent infections, which highlights the importance of this physical barrier to host defenses.

Like the respiratory tract, the digestive tract is a portal of entry through which microbes enter the body, and the mucous membranes lining the digestive tract provide a nonspecific physical barrier against ingested microbes. The intestinal tract is lined with epithelial cells, interspersed with mucus-secreting goblet cells ([link](#)). This mucus mixes with material received from the stomach, trapping foodborne microbes and debris. The mechanical action of **peristalsis**, a series of muscular contractions in the digestive tract, moves the sloughed mucus and other material through the intestines, rectum, and anus, excreting the material in feces.



Goblet cells produce and secrete mucus. The arrows in this micrograph point to the mucus-secreting goblet cells (magnification 1600 $\times$) in the intestinal epithelium. (credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Endothelia

The epithelial cells lining the urogenital tract, blood vessels, lymphatic vessels, and certain other tissues are known as **endothelia**. These tightly packed cells provide a particularly effective frontline barrier against invaders. The endothelia of the **blood-brain barrier**, for example, protect the central nervous system (CNS), which consists of the brain and the spinal cord. The CNS is one of the most sensitive and important areas of the body, as microbial infection of the CNS can quickly lead to serious and often fatal inflammation. The cell junctions in the blood vessels traveling through the

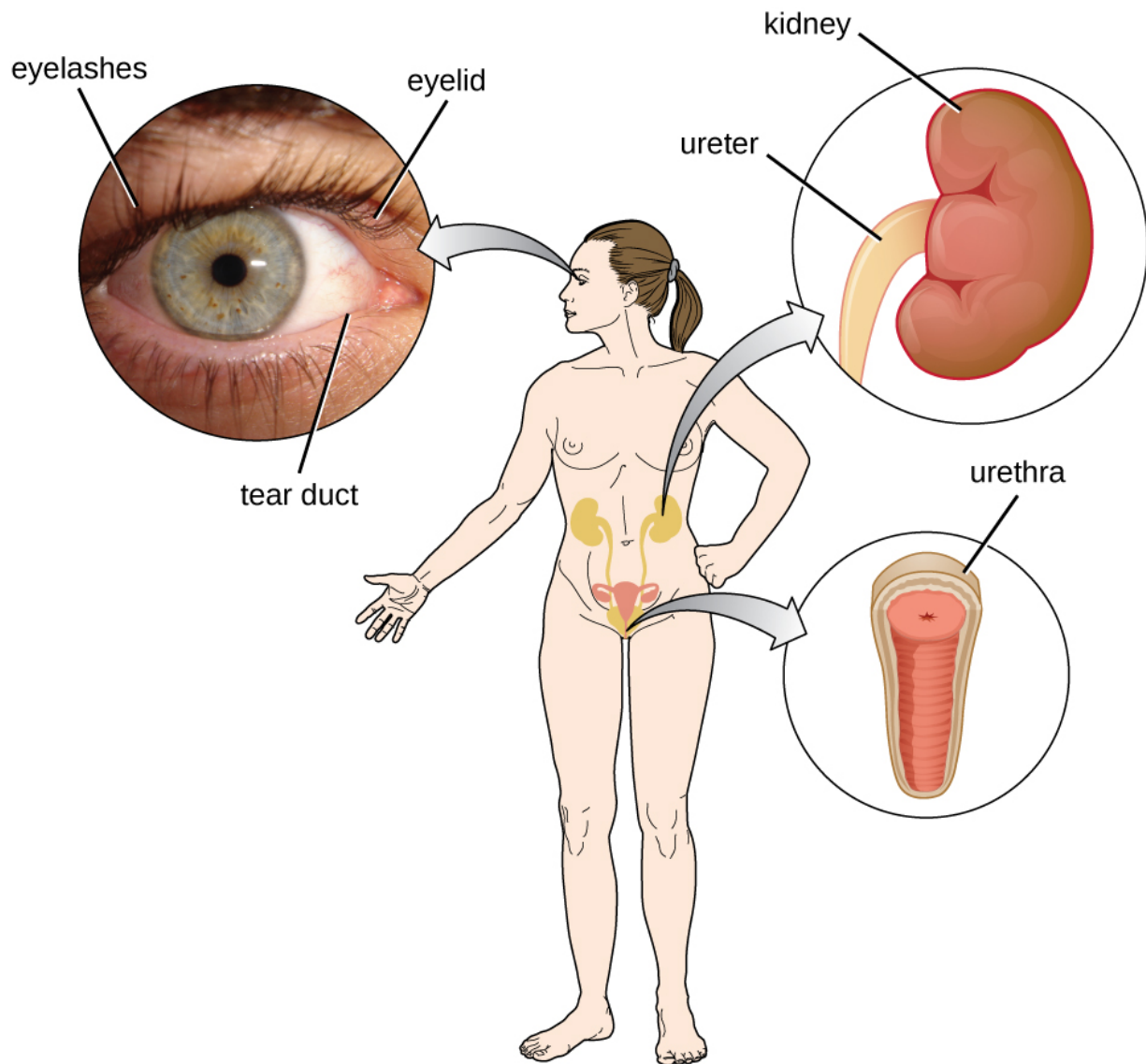
CNS are some of the tightest and toughest in the body, preventing any transient microbes in the bloodstream from entering the CNS. This keeps the cerebrospinal fluid that surrounds and bathes the brain and spinal cord sterile under normal conditions.

Note:

- Describe how the mucociliary escalator functions.
- Name two places you would find endothelia.

Mechanical Defenses

In addition to physical barriers that keep microbes out, the body has a number of mechanical defenses that physically remove pathogens from the body, preventing them from taking up residence. We have already discussed several examples of mechanical defenses, including the shedding of skin cells, the expulsion of mucus via the mucociliary escalator, and the excretion of feces through intestinal peristalsis. Other important examples of mechanical defenses include the flushing action of urine and tears, which both serve to carry microbes away from the body. The flushing action of urine is largely responsible for the normally sterile environment of the urinary tract, which includes the kidneys, ureters, and urinary bladder. Urine passing out of the body washes out transient microorganisms, preventing them from taking up residence. The eyes also have physical barriers and mechanical mechanisms for preventing infections. The eyelashes and eyelids prevent dust and airborne microorganisms from reaching the surface of the eye. Any microbes or debris that make it past these physical barriers may be flushed out by the mechanical action of blinking, which bathes the eye in tears, washing debris away ([\[link\]](#)).



Tears flush microbes away from the surface of the eye. Urine washes microbes out of the urinary tract as it passes through; as a result, the urinary system is normally sterile.

Note:

- Name two mechanical defenses that protect the eyes.

Microbiome

In various regions of the body, resident microbiota serve as an important first-line defense against invading pathogens. Through their occupation of cellular binding sites and competition for available nutrients, the resident microbiota prevent the critical early steps of pathogen attachment and proliferation required for the establishment of an infection. For example, in the vagina, members of the resident microbiota compete with opportunistic pathogens like the yeast *Candida*. This competition prevents infections by limiting the availability of nutrients, thus inhibiting the growth of *Candida*, keeping its population in check. Similar competitions occur between the microbiota and potential pathogens on the skin, in the upper respiratory tract, and in the gastrointestinal tract. As will be discussed later in this chapter, the resident microbiota also contribute to the chemical defenses of the innate nonspecific host defenses.

The importance of the normal microbiota in host defenses is highlighted by the increased susceptibility to infectious diseases when the microbiota is disrupted or eliminated. Treatment with antibiotics can significantly deplete the normal microbiota of the gastrointestinal tract, providing an advantage for pathogenic bacteria to colonize and cause diarrheal infection. In the case of diarrhea caused by *Clostridium difficile*, the infection can be severe and potentially lethal. One strategy for treating *C. difficile* infections is fecal transplantation, which involves the transfer of fecal material from a donor (screened for potential pathogens) into the intestines of the recipient patient as a method of restoring the normal microbiota and combating *C. difficile* infections.

[\[link\]](#) provides a summary of the physical defenses discussed in this section.

Physical Defenses of Nonspecific Innate Immunity

Physical Defense Examples	Nonspecific Innate Immunity	Function
Defense	Examples	Function
Cellular barriers	Skin, mucous membranes, endothelial cells	Deny entry to pathogens
Mechanical defenses	Shedding of skin cells, mucociliary sweeping, peristalsis, flushing action of urine and tears	Remove pathogens from potential sites of infection
Microbiome	Resident bacteria of the skin, upper respiratory tract, gastrointestinal tract, and genitourinary tract	Compete with pathogens for cellular binding sites and nutrients

Note:

- List two ways resident microbiota defend against pathogens.

Key Concepts and Summary

- **Nonspecific innate immunity** provides a first line of defense against infection by nonspecifically blocking entry of microbes and targeting them for destruction or removal from the body.
- The physical defenses of innate immunity include physical barriers, mechanical actions that remove microbes and debris, and the microbiome, which competes with and inhibits the growth of pathogens.

- The skin, mucous membranes, and endothelia throughout the body serve as physical barriers that prevent microbes from reaching potential sites of infection. Tight cell junctions in these tissues prevent microbes from passing through.
- Microbes trapped in dead skin cells or **mucus** are removed from the body by mechanical actions such as shedding of skin cells, mucociliary sweeping, coughing, **peristalsis**, and flushing of bodily fluids (e.g., urination, tears)
- The resident microbiota provide a physical defense by occupying available cellular binding sites and competing with pathogens for available nutrients.

Multiple Choice

Exercise:

Problem:

Which of the following best describes the innate nonspecific immune system?

- A. a targeted and highly specific response to a single pathogen or molecule
- B. a generalized and nonspecific set of defenses against a class or group of pathogens
- C. a set of barrier mechanisms that adapts to specific pathogens after repeated exposure
- D. the production of antibody molecules against pathogens

Solution:

B

Exercise:

Problem:

Which of the following constantly sheds dead cells along with any microbes that may be attached to those cells?

- A. epidermis
- B. dermis
- C. hypodermis
- D. mucous membrane

Solution:

A

Exercise:**Problem:**

Which of the following uses a particularly dense suite of tight junctions to prevent microbes from entering the underlying tissue?

- A. the mucociliary escalator
- B. the epidermis
- C. the blood-brain barrier
- D. the urethra

Solution:

C

Fill in the Blank**Exercise:**

Problem:

The muscular contraction of the intestines that results in movement of material through the digestive tract is called _____.

Solution:

peristalsis

Exercise:**Problem:**

_____ are the hair-like appendages of cells lining parts of the respiratory tract that sweep debris away from the lungs.

Solution:

cilia

Exercise:**Problem:**

Secretions that bathe and moisten the interior of the intestines are produced by _____ cells.

Solution:

goblet

Short Answer**Exercise:****Problem:**

Differentiate a physical barrier from a mechanical removal mechanism and give an example of each.

Exercise:**Problem:**

Identify some ways that pathogens can breach the physical barriers of the innate immune system.

Chemical Defenses

LEARNING OBJECTIVES

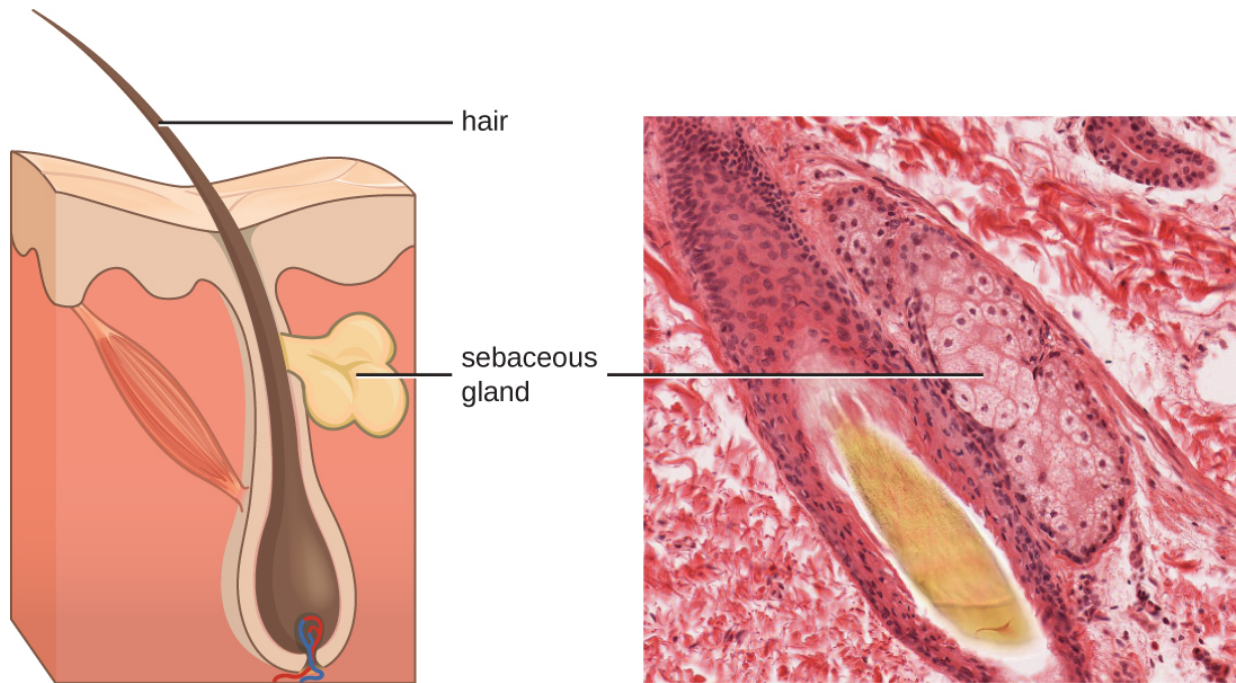
- Describe how enzymes in body fluids provide protection against infection or disease
- List and describe the function of antimicrobial peptides, complement components, cytokines, and acute-phase proteins
- Describe similarities and differences among classic, alternate, and lectin complement pathways

In addition to physical defenses, the innate nonspecific immune system uses a number of **chemical mediators** that inhibit microbial invaders. The term “chemical mediators” encompasses a wide array of substances found in various body fluids and tissues throughout the body. Chemical mediators may work alone or in conjunction with each other to inhibit microbial colonization and infection.

Some chemical mediators are endogenously produced, meaning they are produced by human body cells; others are produced exogenously, meaning that they are produced by certain microbes that are part of the microbiome. Some mediators are produced continually, bathing the area in the antimicrobial substance; others are produced or activated primarily in response to some stimulus, such as the presence of microbes.

Chemical and Enzymatic Mediators Found in Body Fluids

Fluids produced by the skin include examples of both endogenous and exogenous mediators. Sebaceous glands in the dermis secrete an oil called sebum that is released onto the skin surface through hair follicles. This sebum is an endogenous mediator, providing an additional layer of defense by helping seal off the pore of the hair follicle, preventing bacteria on the skin’s surface from invading sweat glands and surrounding tissue ([\[link\]](#)). Certain members of the microbiome, such as the bacterium *Propionibacterium acnes* and the fungus *Malassezia*, among others, can use lipase enzymes to degrade sebum, using it as a food source. This produces oleic acid, which creates a mildly acidic environment on the surface of the skin that is inhospitable to many pathogenic microbes. Oleic acid is an example of an exogenously produced mediator because it is produced by resident microbes and not directly by body cells.



Sebaceous glands secrete sebum, a chemical mediator that lubricates and protect the skin from invading microbes. Sebum is also a food source for resident microbes that produce oleic acid, an exogenously produced mediator. (credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Environmental factors that affect the microbiota of the skin can have a direct impact on the production of chemical mediators. Low humidity or decreased sebum production, for example, could make the skin less habitable for microbes that produce oleic acid, thus making the skin more susceptible to pathogens normally inhibited by the skin's low pH. Many skin moisturizers are formulated to counter such effects by restoring moisture and essential oils to the skin.

The digestive tract also produces a large number of chemical mediators that inhibit or kill microbes. In the oral cavity, saliva contains mediators such as lactoperoxidase enzymes, and mucus secreted by the esophagus contains the antibacterial enzyme lysozyme. In the stomach, highly acidic gastric fluid kills most microbes. In the lower digestive tract, the intestines have pancreatic and intestinal enzymes, antibacterial peptides (cryptins), bile produced from the liver, and specialized Paneth cells that produce lysozyme. Together, these mediators are able to eliminate most pathogens that manage to survive the acidic environment of the stomach.

In the urinary tract, urine flushes microbes out of the body during urination. Furthermore, the slight acidity of urine (the average pH is about 6) inhibits the growth of many microbes

and potential pathogens in the urinary tract.

The female reproductive system employs lactate, an exogenously produced chemical mediator, to inhibit microbial growth. The cells and tissue layers composing the vagina produce glycogen, a branched and more complex polymer of glucose. Lactobacilli in the area ferment glycogen to produce lactate, lowering the pH in the vagina and inhibiting transient microbiota, opportunistic pathogens like *Candida* (a yeast associated with vaginal infections), and other pathogens responsible for sexually transmitted diseases.

In the eyes, tears contain the chemical mediators lysozyme and lactoferrin, both of which are capable of eliminating microbes that have found their way to the surface of the eyes. Lysozyme cleaves the bond between NAG and NAM in peptidoglycan, a component of the cell wall in bacteria. It is more effective against gram-positive bacteria, which lack the protective outer membrane associated with gram-negative bacteria. Lactoferrin inhibits microbial growth by chemically binding and sequestering iron. This effectually starves many microbes that require iron for growth.

In the ears, cerumen (earwax) exhibits antimicrobial properties due to the presence of fatty acids, which lower the pH to between 3 and 5.

The respiratory tract uses various chemical mediators in the nasal passages, trachea, and lungs. The mucus produced in the nasal passages contains a mix of antimicrobial molecules similar to those found in tears and saliva (e.g., lysozyme, lactoferrin, lactoperoxidase). Secretions in the trachea and lungs also contain lysozyme and lactoferrin, as well as a diverse group of additional chemical mediators, such as the lipoprotein complex called surfactant, which has antibacterial properties.

Note:

- Explain the difference between endogenous and exogenous mediators
- Describe how pH affects antimicrobial defenses

Antimicrobial Peptides

The **antimicrobial peptides (AMPs)** are a special class of nonspecific cell-derived mediators with broad-spectrum antimicrobial properties. Some AMPs are produced routinely by the body, whereas others are primarily produced (or produced in greater quantities) in response to the presence of an invading pathogen. Research has begun exploring how AMPs can be used in the diagnosis and treatment of disease.

AMPs may induce cell damage in microorganisms in a variety of ways, including by inflicting damage to membranes, destroying DNA and RNA, or interfering with cell-wall synthesis. Depending on the specific antimicrobial mechanism, a particular AMP may inhibit only certain groups of microbes (e.g., gram-positive or gram-negative bacteria) or it may be more broadly effective against bacteria, fungi, protozoa, and viruses. Many AMPs are found on the skin, but they can also be found in other regions of the body.

A family of AMPs called defensins can be produced by epithelial cells throughout the body as well as by cellular defenses such as macrophages and neutrophils (see [Cellular Defenses](#)). Defensins may be secreted or act inside host cells; they combat microorganisms by damaging their plasma membranes. AMPs called bacteriocins are produced exogenously by certain members of the resident microbiota within the gastrointestinal tract. The genes coding for these types of AMPs are often carried on plasmids and can be passed between different species within the resident microbiota through lateral or horizontal gene transfer.

There are numerous other AMPs throughout the body. The characteristics of a few of the more significant AMPs are summarized in [\[link\]](#).

Characteristics of Selected Antimicrobial Peptides (AMPs)				
AMP	Secreted by	Body site	Pathogens inhibited	Mode of action
Bacteriocins	Resident microbiota	Gastrointestinal tract	Bacteria	Disrupt membrane
Cathelicidin	Epithelial cells, macrophages, and other cell types	Skin	Bacteria and fungi	Disrupts membrane
Defensins	Epithelial cells, macrophages, neutrophils	Throughout the body	Fungi, bacteria, and many viruses	Disrupt membrane

Characteristics of Selected Antimicrobial Peptides (AMPs)				
AMP	Secreted by	Body site	Pathogens inhibited	Mode of action
Dermicidin	Sweat glands	Skin	Bacteria and fungi	Disrupts membrane integrity and ion channels
Histatins	Salivary glands	Oral cavity	Fungi	Disrupt intracellular function

Note:

- Why are antimicrobial peptides (AMPs) considered nonspecific defenses?

Plasma Protein Mediators

Many nonspecific innate immune factors are found in **plasma**, the fluid portion of blood. Plasma contains electrolytes, sugars, lipids, and proteins, each of which helps to maintain homeostasis (i.e., stable internal body functioning), and contains the proteins involved in the clotting of blood. Additional proteins found in blood plasma, such as acute-phase proteins, complement proteins, and cytokines, are involved in the nonspecific innate immune response.

Note:

Plasma versus Serum

There are two terms for the fluid portion of blood: plasma and serum. How do they differ if they are both fluid and lack cells? The fluid portion of blood left over after coagulation (blood cell clotting) has taken place is serum. Although molecules such as many vitamins, electrolytes, certain sugars, complement proteins, and antibodies are still present in serum, clotting factors are largely depleted. Plasma, conversely, still contains all the clotting elements. To obtain plasma from blood, an anticoagulant must be used to prevent clotting. Examples of anticoagulants include heparin and ethylene diamine tetraacetic acid (EDTA).

Because clotting is inhibited, once obtained, the sample must be gently spun down in a centrifuge. The heavier, denser blood cells form a pellet at the bottom of a centrifuge tube, while the fluid plasma portion, which is lighter and less dense, remains above the cell pellet.

Acute-Phase Proteins

The **acute-phase proteins** are another class of antimicrobial mediators. Acute-phase proteins are primarily produced in the liver and secreted into the blood in response to inflammatory molecules from the immune system. Examples of acute-phase proteins include C-reactive protein, serum amyloid A, ferritin, transferrin, fibrinogen, and mannose-binding lectin. Each of these proteins has a different chemical structure and inhibits or destroys microbes in some way ([\[link\]](#)).

Some Acute-Phase Proteins and Their Functions	
C-reactive protein	Coats bacteria (opsonization), preparing them for ingestion by phagocytes
Serum amyloid A	
Ferritin	Bind and sequester iron, thereby inhibiting the growth of pathogens
Transferrin	
Fibrinogen	Involved in formation of blood clots that trap bacterial pathogens
Mannose-binding lectin	Activates complement cascade

The Complement System

The **complement system** is a group of plasma protein mediators that can act as an innate nonspecific defense while also serving to connect innate and adaptive immunity (discussed in the next chapter). The complement system is composed of more than 30 proteins (including C1 through C9) that normally circulate as precursor proteins in blood. These

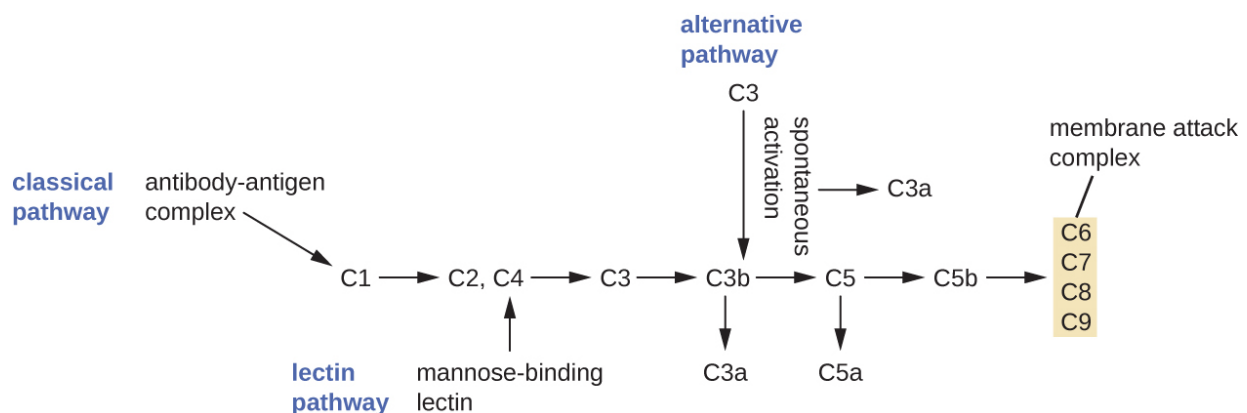
precursor proteins become activated when stimulated or triggered by a variety of factors, including the presence of microorganisms. Complement proteins are considered part of innate nonspecific immunity because they are always present in the blood and tissue fluids, allowing them to be activated quickly. Also, when activated through the alternative pathway (described later in this section), complement proteins target pathogens in a nonspecific manner.

The process by which circulating complement precursors become functional is called **complement activation**. This process is a cascade that can be triggered by one of three different mechanisms, known as the alternative, classical, and lectin pathways.

The alternative pathway is initiated by the spontaneous activation of the complement protein C3. The hydrolysis of C3 produces two products, C3a and C3b. When no invader microbes are present, C3b is very quickly degraded in a hydrolysis reaction using the water in the blood. However, if invading microbes are present, C3b attaches to the surface of these microbes. Once attached, C3b will recruit other complement proteins in a cascade ([\[link\]](#)).

The classical pathway provides a more efficient mechanism of activating the complement cascade, but it depends upon the production of antibodies by the specific adaptive immune defenses. To initiate the classical pathway, a specific antibody must first bind to the pathogen to form an antibody-antigen complex. This activates the first protein in the complement cascade, the C1 complex. The C1 complex is a multipart protein complex, and each component participates in the full activation of the overall complex. Following recruitment and activation of the C1 complex, the remaining classical pathway complement proteins are recruited and activated in a cascading sequence ([\[link\]](#)).

The lectin activation pathway is similar to the classical pathway, but it is triggered by the binding of mannose-binding lectin, an acute-phase protein, to carbohydrates on the microbial surface. Like other acute-phase proteins, lectins are produced by liver cells and are commonly upregulated in response to inflammatory signals received by the body during an infection ([\[link\]](#)).



The three complement activation pathways have different triggers, as shown here, but all three result in the activation of the complement protein C3, which produces C3a and C3b. The latter binds to the surface of the target cell and then works with other complement proteins to cleave C5 into C5a and C5b. C5b also binds to the cell surface and then recruits C6 through C9; these molecules form a ring structure called the membrane attack complex (MAC), which punches through the cell membrane of the invading pathogen, causing it to swell and burst.

Although each complement activation pathway is initiated in a different way, they all provide the same protective outcomes: opsonization, inflammation, chemotaxis, and cytolysis. The term **opsonization** refers to the coating of a pathogen by a chemical substance (called an **opsonin**) that allows phagocytic cells to recognize, engulf, and destroy it more easily. Opsonins from the complement cascade include C1q, C3b, and C4b. Additional important opsonins include mannose-binding proteins and antibodies. The complement fragments C3a and C5a are well-characterized anaphylatoxins with potent proinflammatory functions. Anaphylatoxins activate mast cells, causing degranulation and the release of inflammatory chemical signals, including mediators that cause vasodilation and increased vascular permeability. C5a is also one of the most potent chemoattractants for neutrophils and other white blood cells, cellular defenses that will be discussed in the next section.

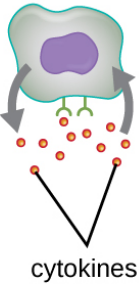
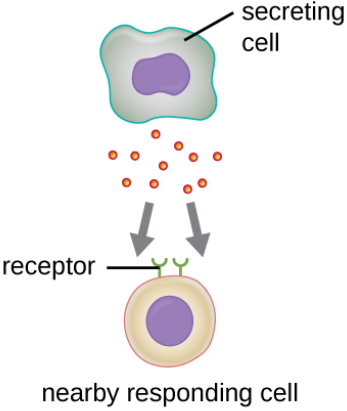
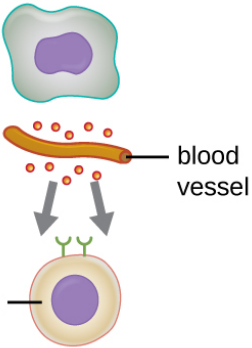
The complement proteins C6, C7, C8, and C9 assemble into a **membrane attack complex (MAC)**, which allows C9 to polymerize into pores in the membranes of gram-negative bacteria. These pores allow water, ions, and other molecules to move freely in and out of the targeted cells, eventually leading to cell lysis and death of the pathogen ([\[link\]](#)). However, the MAC is only effective against gram-negative bacteria; it cannot penetrate the thick layer of peptidoglycan associated with cell walls of gram-positive bacteria. Since the MAC does not pose a lethal threat to gram-positive bacterial pathogens, complement-mediated opsonization is more important for their clearance.

Cytokines

Cytokines are soluble proteins that act as communication signals between cells. In a nonspecific innate immune response, various cytokines may be released to stimulate production of chemical mediators or other cell functions, such as cell proliferation, cell differentiation, inhibition of cell division, apoptosis, and chemotaxis.

When a cytokine binds to its target receptor, the effect can vary widely depending on the type of cytokine and the type of cell or receptor to which it has bound. The function of a particular cytokine can be described as autocrine, paracrine, or endocrine ([\[link\]](#)). In **autocrine function**, the same cell that releases the cytokine is the recipient of the signal; in

other words, autocrine function is a form of self-stimulation by a cell. In contrast, **paracrine function** involves the release of cytokines from one cell to other nearby cells, stimulating some response from the recipient cells. Last, **endocrine function** occurs when cells release cytokines into the bloodstream to be carried to target cells much farther away.

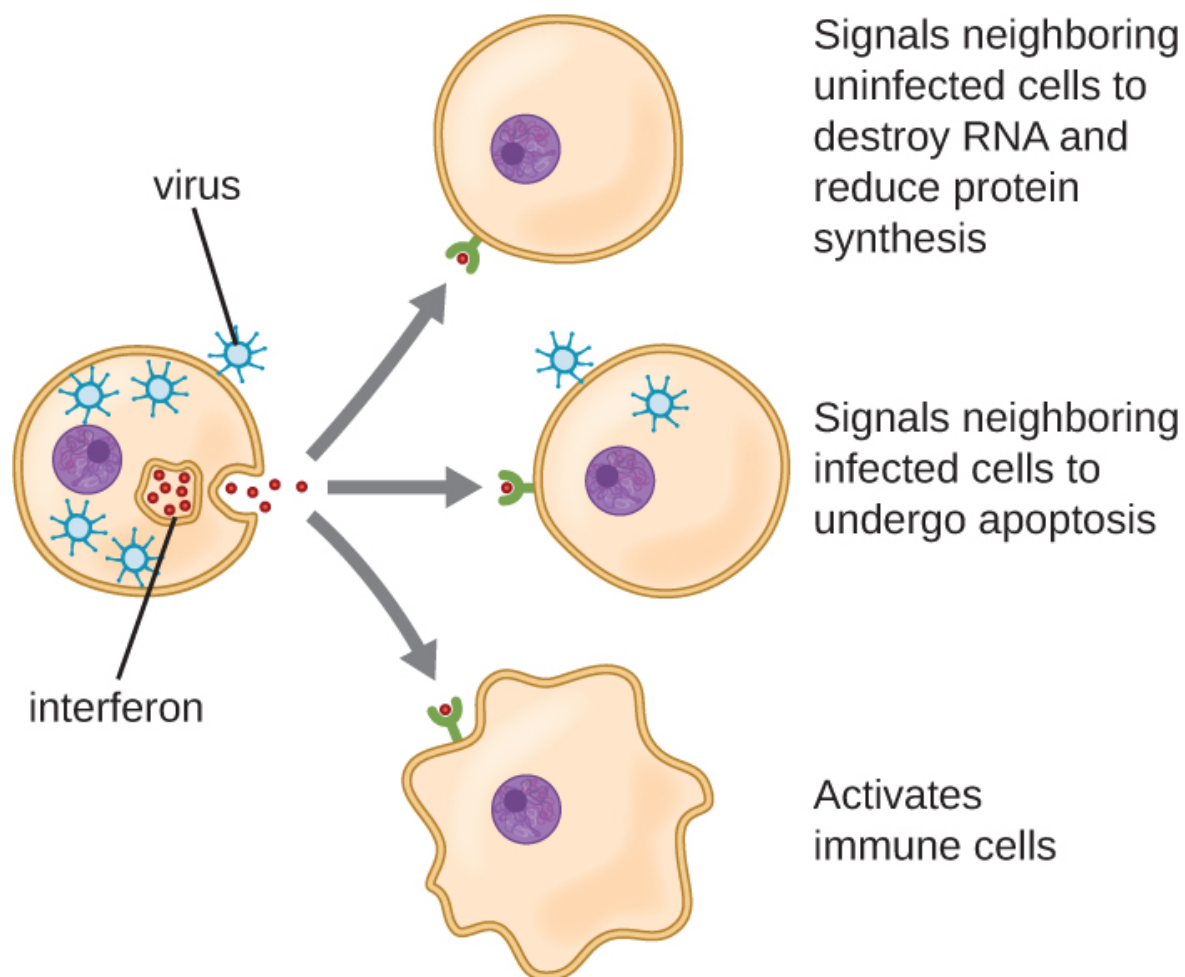
CYTOKINES: Molecular Messengers		
Autocrine	Paracrine	Endocrine
Same cell secretes and receives cytokine signal.	Cytokine signal secreted to a nearby cell.	Cytokine signal secreted to circulatory system; travels to distant cells.
		

Autocrine, paracrine, and endocrine actions describe which cells are targeted by cytokines and how far the cytokines must travel to bind to their intended target cells’ receptors.

Three important classes of cytokines are the interleukins, chemokines, and interferons. The **interleukins** were originally thought to be produced only by leukocytes (white blood cells) and to only stimulate leukocytes, thus the reasons for their name. Although interleukins are involved in modulating almost every function of the immune system, their role in the body is not restricted to immunity. Interleukins are also produced by and stimulate a variety of cells unrelated to immune defenses.

The **chemokines** are chemotactic factors that recruit leukocytes to sites of infection, tissue damage, and inflammation. In contrast to more general chemotactic factors, like complement factor C5a, chemokines are very specific in the subsets of leukocytes they recruit.

Interferons are a diverse group of immune signaling molecules and are especially important in our defense against viruses. Type I **interferons** (interferon- α and interferon- β) are produced and released by cells infected with virus. These interferons stimulate nearby cells to stop production of mRNA, destroy RNA already produced, and reduce protein synthesis. These cellular changes inhibit viral replication and production of mature virus, slowing the spread of the virus. Type I interferons also stimulate various immune cells involved in viral clearance to more aggressively attack virus-infected cells. Type II interferon (interferon- γ) is an important activator of immune cells ([link](#)).



Interferons are cytokines released by a cell infected with a virus. Interferon- α and interferon- β signal uninfected neighboring cells to inhibit mRNA synthesis, destroy RNA, and reduce protein synthesis (top arrow). Interferon- α and interferon- β also promote apoptosis in cells infected with the virus (middle arrow). Interferon- γ alerts neighboring immune cells to an attack (bottom arrow). Although interferons do not cure the cell releasing them or other infected cells,

which will soon die, their release may prevent additional cells from becoming infected, thus stemming the infection.

Inflammation-Eliciting Mediators

Many of the chemical mediators discussed in this section contribute in some way to inflammation and fever, which are nonspecific immune responses discussed in more detail in [Inflammation and Fever](#). Cytokines stimulate the production of acute-phase proteins such as C-reactive protein and mannose-binding lectin in the liver. These acute-phase proteins act as opsonins, activating complement cascades through the lectin pathway.

Some cytokines also bind mast cells and basophils, inducing them to release **histamine**, a proinflammatory compound. Histamine receptors are found on a variety of cells and mediate proinflammatory events, such as bronchoconstriction (tightening of the airways) and smooth muscle contraction.

In addition to histamine, mast cells may release other chemical mediators, such as **leukotrienes**. Leukotrienes are lipid-based proinflammatory mediators that are produced from the metabolism of arachidonic acid in the cell membrane of leukocytes and tissue cells. Compared with the proinflammatory effects of histamine, those of leukotrienes are more potent and longer lasting. Together, these chemical mediators can induce coughing, vomiting, and diarrhea, which serve to expel pathogens from the body.

Certain cytokines also stimulate the production of prostaglandins, chemical mediators that promote the inflammatory effects of kinins and histamines. Prostaglandins can also help to set the body temperature higher, leading to fever, which promotes the activities of white blood cells and slightly inhibits the growth of pathogenic microbes (see [Inflammation and Fever](#)).

Another inflammatory mediator, **bradykinin**, contributes to edema, which occurs when fluids and leukocytes leak out of the bloodstream and into tissues. It binds to receptors on cells in the capillary walls, causing the capillaries to dilate and become more permeable to fluids.

Note:

- What do the three complement activation pathways have in common?
- Explain autocrine, paracrine, and endocrine signals.
- Name two important inflammation-eliciting mediators.

Note:**Part 2**

To relieve the constriction of her airways, Angela is immediately treated with antihistamines and administered corticosteroids through an inhaler, and then monitored for a period of time. Though her condition does not worsen, the drugs do not seem to be alleviating her condition. She is admitted to the hospital for further observation, testing, and treatment.

Following admission, a clinician conducts allergy testing to try to determine if something in her environment might be triggering an allergic inflammatory response. A doctor orders blood analysis to check for levels of particular cytokines. A sputum sample is also taken and sent to the lab for microbial staining, culturing, and identification of pathogens that could be causing an infection.

- Which aspects of the innate immune system could be contributing to Angela's airway constriction?
- Why was Angela treated with antihistamines?
- Why would the doctor be interested in levels of cytokines in Angela's blood?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

[\[link\]](#) provides a summary of the chemical defenses discussed in this section.

Chemical Defenses of Nonspecific Innate Immunity		
Defense	Examples	Function
Chemicals and enzymes in body fluids	Sebum from sebaceous glands	Provides oil barrier protecting hair follicle pores from pathogens
	Oleic acid from sebum and skin microbiota	Lowers pH to inhibit pathogens
	Lysozyme in secretions	Kills bacteria by attacking cell wall
	Acid in stomach, urine, and vagina	Inhibits or kills bacteria

Chemical Defenses of Nonspecific Innate Immunity		
Defense	Examples	Function
	Digestive enzymes and bile	Kill bacteria
	Lactoferrin and transferrin	Bind and sequester iron, inhibiting bacterial growth
	Surfactant in lungs	Kills bacteria
Antimicrobial peptides	Defensins, bacteriocins, dermicidin, cathelicidin, histatins,	Kill bacteria by attacking membranes or interfering with cell functions
Plasma protein mediators	Acute-phase proteins (C-reactive protein, serum amyloid A, ferritin, fibrinogen, transferrin, and mannose-binding lectin)	Inhibit the growth of bacteria and assist in the trapping and killing of bacteria
	Complements C3b and C4b	Opsonization of pathogens to aid phagocytosis
	Complement C5a	Chemoattractant for phagocytes
	Complements C3a and C5a	Proinflammatory anaphylatoxins
Cytokines	Interleukins	Stimulate and modulate most functions of immune system
	Chemokines	Recruit white blood cells to infected area

Chemical Defenses of Nonspecific Innate Immunity		
Defense	Examples	Function
	Interferons	Alert cells to viral infection, induce apoptosis of virus-infected cells, induce antiviral defenses in infected and nearby uninfected cells, stimulate immune cells to attack virus-infected cells
Inflammation-eliciting mediators	Histamine	Promotes vasodilation, bronchoconstriction, smooth muscle contraction, increased secretion and mucus production
	Leukotrienes	Promote inflammation; stronger and longer lasting than histamine
	Prostaglandins	Promote inflammation and fever
	Bradykinin	Increases vasodilation and vascular permeability, leading to edema

Key Concepts and Summary

- Numerous **chemical mediators** produced endogenously and exogenously exhibit nonspecific antimicrobial functions.
- Many chemical mediators are found in body fluids such as sebum, saliva, mucus, gastric and intestinal fluids, urine, tears, cerumen, and vaginal secretions.
- **Antimicrobial peptides (AMPs)** found on the skin and in other areas of the body are largely produced in response to the presence of pathogens. These include dermcidin, cathelicidin, defensins, histatins, and bacteriocins.
- **Plasma** contains various proteins that serve as chemical mediators, including **acute-phase proteins, complement proteins, and cytokines**.
- The **complement system** involves numerous precursor proteins that circulate in plasma. These proteins become activated in a cascading sequence in the presence of microbes, resulting in the **opsonization** of pathogens, chemoattraction of leukocytes, induction of inflammation, and cytolysis through the formation of a **membrane attack complex (MAC)**.
- **Cytokines** are proteins that facilitate various nonspecific responses by innate immune cells, including production of other chemical mediators, cell proliferation, cell death, and differentiation.

- Cytokines play a key role in the inflammatory response, triggering production of inflammation-eliciting mediators such as acute-phase proteins, **histamine**, leukotrienes, **prostaglandins**, and **bradykinin**.

Multiple Choice

Exercise:

Problem:

Which of the following serve as chemical signals between cells and stimulate a wide range of nonspecific defenses?

- A. cytokines
- B. antimicrobial peptides
- C. complement proteins
- D. antibodies

Solution:

A

Exercise:

Problem: Bacteriocins and defensins are types of which of the following?

- A. leukotrienes
- B. cytokines
- C. inflammation-eliciting mediators
- D. antimicrobial peptides

Solution:

D

Exercise:

Problem:

Which of the following chemical mediators is secreted onto the surface of the skin?

- A. cerumen
- B. sebum
- C. gastric acid
- D. prostaglandin

Solution:

B

Exercise:

Problem:

Identify the complement activation pathway that is triggered by the binding of an acute-phase protein to a pathogen.

- A. classical
- B. alternate
- C. lectin
- D. cathelicidin

Solution:

C

Exercise:

Problem:

Histamine, leukotrienes, prostaglandins, and bradykinin are examples of which of the following?

- A. chemical mediators primarily found in the digestive system
- B. chemical mediators that promote inflammation
- C. antimicrobial peptides found on the skin
- D. complement proteins that form MACs

Solution:

B

Fill in the Blank

Exercise:

Problem:

_____ are antimicrobial peptides produced by members of the normal microbiota.

Solution:

bacteriocins

Exercise:

Problem:

_____ is the fluid portion of a blood sample that has been drawn in the presence of an anticoagulant compound.

Solution:

plasma

Exercise:

Problem:

The process by which cells are drawn or attracted to an area by a microbe invader is known as _____.

Solution:

chemotaxis

Short Answer

Exercise:

Problem:

Differentiate the main activation methods of the classic, alternative, and lectin complement cascades.

Exercise:

Problem: What are the four protective outcomes of complement activation?

LEARNING OBJECTIVES

- Identify and describe the components of blood
- Explain the process by which the formed elements of blood are formed (hematopoiesis)
- Describe the characteristics of formed elements found in peripheral blood, as well as their respective functions within the innate immune system

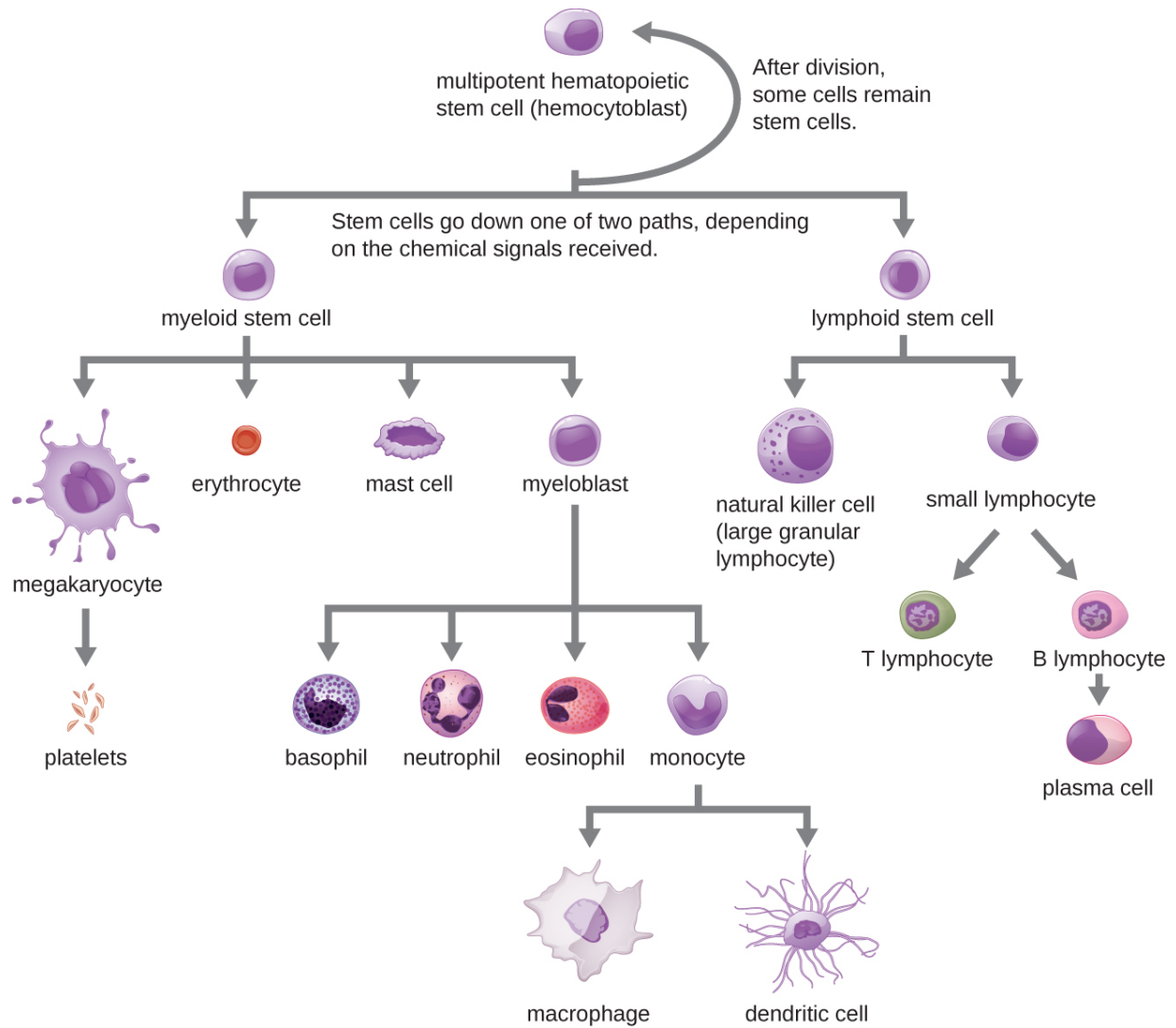
In the previous section, we discussed some of the chemical mediators found in plasma, the fluid portion of blood. The nonfluid portion of blood consists of various types of formed elements, so called because they are all formed from the same stem cells found in bone marrow. The three major categories of formed elements are: red blood cells (RBCs), also called **erythrocytes**; **platelets**, also called **thrombocytes**; and white blood cells (WBCs), also called **leukocytes**.

Red blood cells are primarily responsible for carrying oxygen to tissues. Platelets are cellular fragments that participate in blood clot formation and tissue repair. Several different types of WBCs participate in various nonspecific mechanisms of innate and adaptive immunity. In this section, we will focus primarily on the innate mechanisms of various types of WBCs.

Hematopoiesis

All of the formed elements of blood are derived from pluripotent hematopoietic stem cells (HSCs) in the bone marrow. As the HSCs make copies of themselves in the bone marrow, individual cells receive different cues from the body that control how they develop and mature. As a result, the HSCs differentiate into different types of blood cells that, once mature, circulate in peripheral blood. This process of differentiation, called **hematopoiesis**, is shown in more detail in [\[link\]](#).

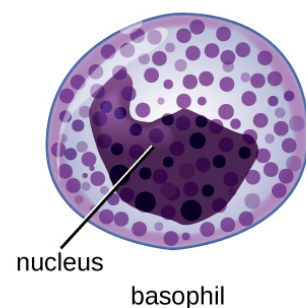
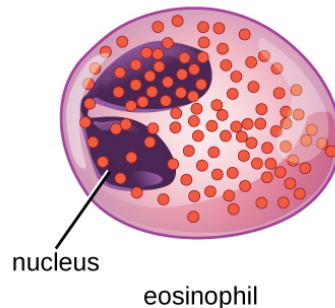
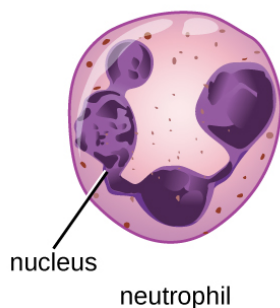
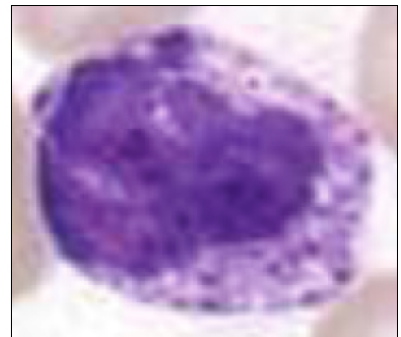
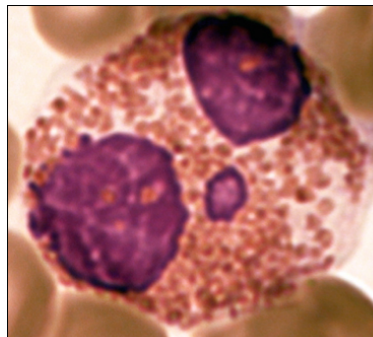
In terms of sheer numbers, the vast majority of HSCs become erythrocytes. Much smaller numbers become leukocytes and platelets. Leukocytes can be further subdivided into **granulocytes**, which are characterized by numerous granules visible in the cytoplasm, and agranulocytes, which lack granules. [\[link\]](#) provides an overview of the various types of formed elements, including their relative numbers, primary function, and lifespans.



All the formed elements of the blood arise by differentiation of hematopoietic stem cells in the bone marrow.

Granulocytes

The various types of granulocytes can be distinguished from one another in a blood smear by the appearance of their nuclei and the contents of their granules, which confer different traits, functions, and staining properties. The **neutrophils**, also called **polymorphonuclear neutrophils (PMNs)**, have a nucleus with three to five lobes and small, numerous, lilac-colored granules. Each lobe of the nucleus is connected by a thin strand of material to the other lobes. The **eosinophils** have fewer lobes in the nucleus (typically 2–3) and larger granules that stain reddish-orange. The **basophils** have a two-lobed nucleus and large granules that stain dark blue or purple ([\[link\]](#)).



Granulocytes can be distinguished by the number of lobes in their nuclei and the staining properties of their granules. (credit “neutrophil” micrograph: modification of work by Ed Uthman)

Neutrophils (PMNs)

Neutrophils (PMNs) are frequently involved in the elimination and destruction of extracellular bacteria. They are capable of migrating through the walls of blood vessels to areas of bacterial infection and tissue damage, where they seek out and kill infectious bacteria. PMN granules contain a variety of defensins and hydrolytic enzymes that help them destroy bacteria through phagocytosis (described in more detail in [Pathogen Recognition and Phagocytosis](#)) In addition, when many neutrophils are brought into an infected area, they can be stimulated to release toxic molecules into the surrounding tissue to better clear infectious agents. This is called degranulation.

Another mechanism used by neutrophils is neutrophil extracellular traps (NETs), which are extruded meshes of chromatin that are closely associated with antimicrobial granule proteins and components. Chromatin is DNA with associated proteins (usually histone proteins, around which DNA wraps for organization and packing within a cell). By creating and releasing a mesh or lattice-like structure of chromatin that is coupled with antimicrobial proteins, the neutrophils can mount a highly concentrated and efficient attack against nearby pathogens. Proteins frequently associated with NETs include lactoferrin, gelatinase, cathepsin G, and myeloperoxidase. Each has a different means of promoting antimicrobial activity, helping neutrophils eliminate pathogens. The toxic proteins in NETs may kill some of the body's own cells along with invading pathogens. However, this collateral damage can be repaired after the danger of the infection has been eliminated.

As neutrophils fight an infection, a visible accumulation of leukocytes, cellular debris, and bacteria at the site of infection can be observed. This buildup is what we call **pus** (also known as purulent or suppurative discharge or drainage). The presence of pus is a sign that the immune defenses have been activated against an infection; historically, some physicians believed that inducing pus formation could actually promote the healing of wounds. The practice of promoting “laudable pus” (by, for

instance, wrapping a wound in greasy wool soaked in wine) dates back to the ancient physician Galen in the 2nd century AD, and was practiced in variant forms until the 17th century (though it was not universally accepted). Today, this method is no longer practiced because we now know that it is not effective. Although a small amount of pus formation can indicate a strong immune response, artificially inducing pus formation does not promote recovery.

Eosinophils

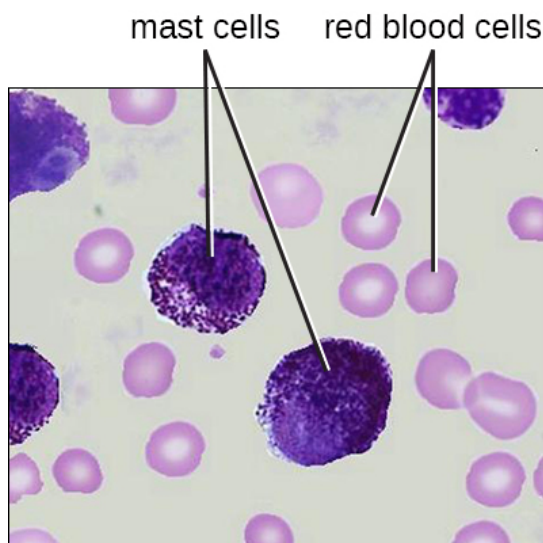
Eosinophils are granulocytes that protect against protozoa and helminths; they also play a role in allergic reactions. The granules of eosinophils, which readily absorb the acidic reddish dye eosin, contain histamine, degradative enzymes, and a compound known as major basic protein (MBP) ([\[link\]](#)). MBP binds to the surface carbohydrates of parasites, and this binding is associated with disruption of the cell membrane and membrane permeability.

Basophils

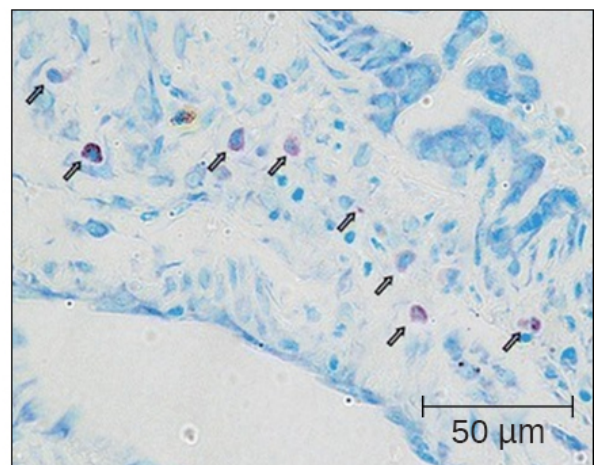
Basophils have cytoplasmic granules of varied size and are named for their granules' ability to absorb the basic dye methylene blue ([\[link\]](#)). Their stimulation and degranulation can result from multiple triggering events. Activated complement fragments C3a and C5a, produced in the activation cascades of complement proteins, act as anaphylatoxins by inducing degranulation of basophils and inflammatory responses. This cell type is important in allergic reactions and other responses that involve inflammation. One of the most abundant components of basophil granules is histamine, which is released along with other chemical factors when the basophil is stimulated. These chemicals can be chemotactic and can help to open the gaps between cells in the blood vessels. Other mechanisms for basophil triggering require the assistance of antibodies, as discussed in [B Lymphocytes and Humoral Immunity](#).

Mast Cells

Hematopoiesis also gives rise to **mast cells**, which appear to be derived from the same common myeloid progenitor cell as neutrophils, eosinophils, and basophils. Functionally, mast cells are very similar to basophils, containing many of the same components in their granules (e.g., histamine) and playing a similar role in allergic responses and other inflammatory reactions. However, unlike basophils, mast cells leave the circulating blood and are most frequently found residing in tissues. They are often associated with blood vessels and nerves or found close to surfaces that interface with the external environment, such as the skin and mucous membranes in various regions of the body ([link](#)).



(a)



(b)

Mast cells function similarly to basophils by inducing and promoting inflammatory responses. (a) This figure shows mast cells in blood. In a blood smear, they are difficult to differentiate from basophils (b).

Unlike basophils, mast cells migrate from the blood into various tissues. (credit right: modification of work by Greenland JR, Xu X, Sayah DM, Liu FC, Jones KD, Looney MR, Caughey GH)

Note:

- Describe the granules and nuclei of neutrophils, eosinophils, basophils, and mast cells.
- Name three antimicrobial mechanisms of neutrophils

Note:**Part 3**

Angela's tests come back negative for all common allergens, and her sputum samples contain no abnormal presence of pathogenic microbes or elevated levels of members of the normal respiratory microbiota. She does, however, have elevated levels of inflammatory cytokines in her blood. The swelling of her airway has still not responded to treatment with antihistamines or corticosteroids. Additional blood work shows that Angela has a mildly elevated white blood cell count but normal antibody levels. Also, she has a lower-than-normal level of the complement protein C4.

- What does this new information reveal about the cause of Angela's constricted airways?
- What are some possible conditions that could lead to low levels of complement proteins?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Agranulocytes

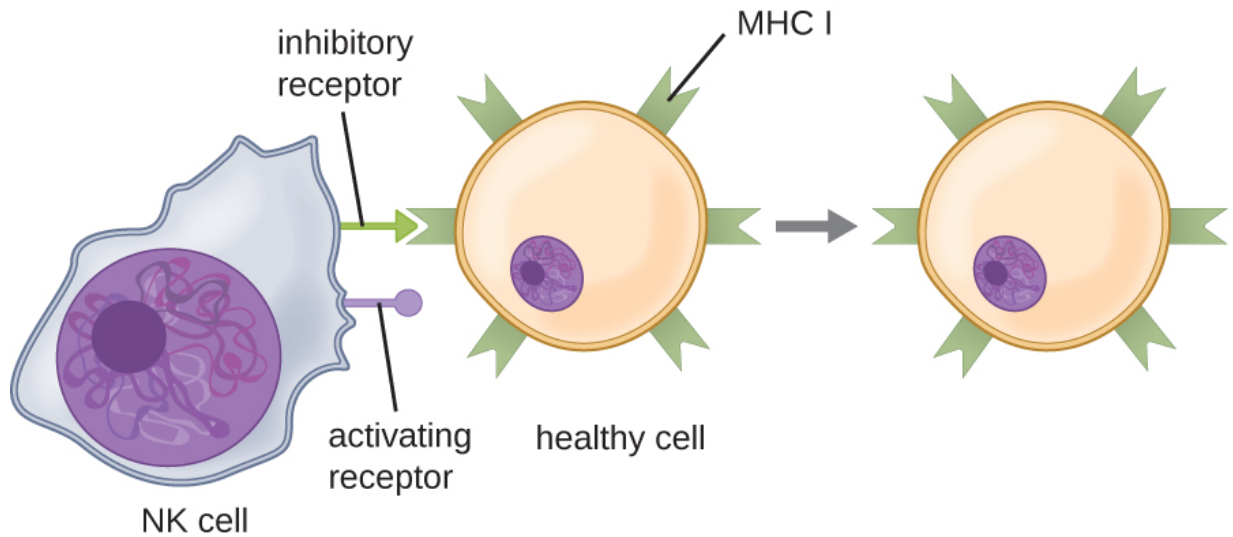
As their name suggests, **agranulocytes** lack visible granules in the cytoplasm. Agranulocytes can be categorized as lymphocytes or monocytes ([link](#)). Among the lymphocytes are natural killer cells, which play an important role in nonspecific innate immune defenses. Lymphocytes also include the B cells and T cells, which are discussed in the next chapter

because they are central players in the specific adaptive immune defenses. The monocytes differentiate into macrophages and dendritic cells, which are collectively referred to as the mononuclear phagocyte system.

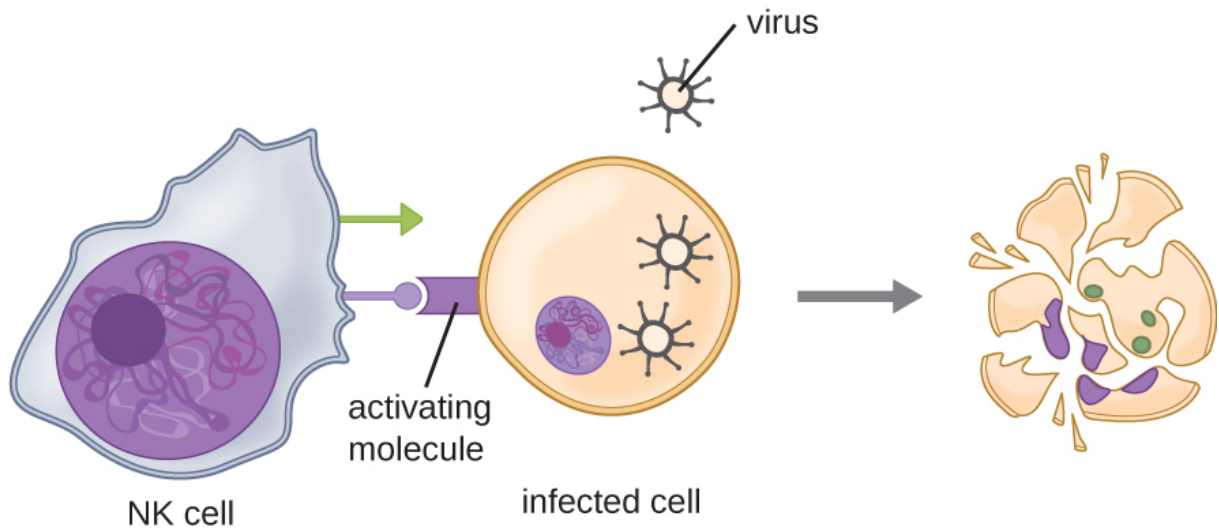
Natural Killer Cells

Most lymphocytes are primarily involved in the specific adaptive immune response, and thus will be discussed in the following chapter. An exception is the **natural killer cells (NK cells)**; these mononuclear lymphocytes use nonspecific mechanisms to recognize and destroy cells that are abnormal in some way. Cancer cells and cells infected with viruses are two examples of cellular abnormalities that are targeted by NK cells. Recognition of such cells involves a complex process of identifying inhibitory and activating molecular markers on the surface of the target cell. Molecular markers that make up the major histocompatibility complex (MHC) are expressed by healthy cells as an indication of “self.” This will be covered in more detail in next chapter. NK cells are able to recognize normal MHC markers on the surface of healthy cells, and these MHC markers serve as an inhibitory signal preventing NK cell activation. However, cancer cells and virus-infected cells actively diminish or eliminate expression of MHC markers on their surface. When these MHC markers are diminished or absent, the NK cell interprets this as an abnormality and a cell in distress. This is one part of the NK cell activation process ([\[link\]](#)). NK cells are also activated by binding to activating molecular molecules on the target cell. These activating molecular molecules include “altered self” or “nonself” molecules. When a NK cell recognizes a decrease in inhibitory normal MHC molecules and an increase in activating molecules on the surface of a cell, the NK cell will be activated to eliminate the cell in distress.

A natural killer (NK) cell recognizes MHC I on a healthy cell and does not kill it.



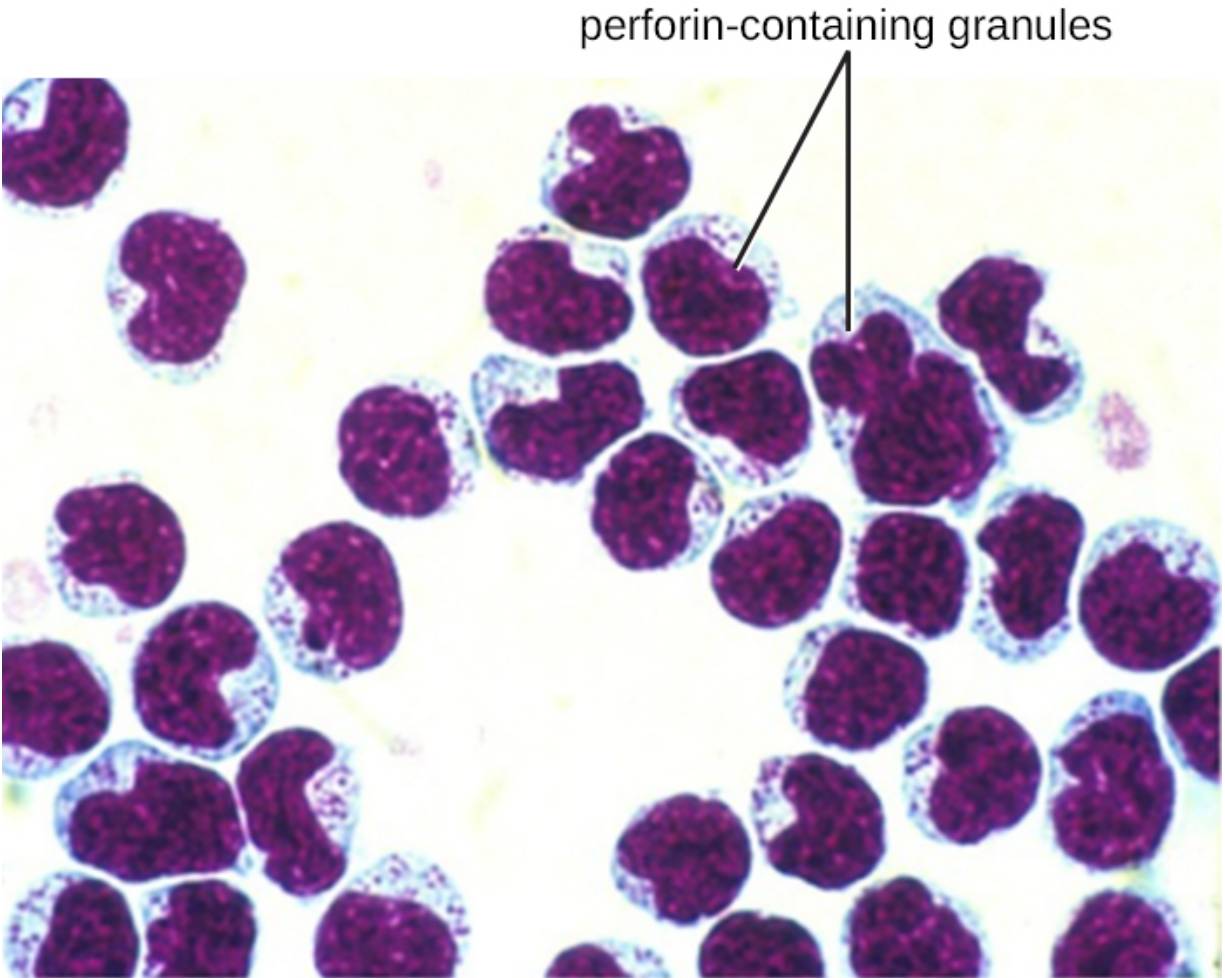
An infected cell does not present the MHC I, but does present ligands for the activating receptor. The NK cell will trigger a response that kills this cell.



Natural killer (NK) cells are inhibited by the presence of the major histocompatibility cell (MHC) receptor on healthy cells. Cancer cells and virus-infected cells have reduced expression of MHC and increased expression of activating molecules. When a NK cell recognizes decreased MHC and increased activating molecules, it will kill the abnormal cell.

Once a cell has been recognized as a target, the NK cell can use several different mechanisms to kill its target. For example, it may express cytotoxic membrane proteins and cytokines that stimulate the target cell to undergo apoptosis, or controlled cell suicide. NK cells may also use perforin-mediated cytotoxicity to induce apoptosis in target cells. This mechanism relies on two toxins released from granules in the cytoplasm of the NK cell: **perforin**, a protein that creates pores in the target cell, and **granzymes**, proteases that enter through the pores into the target cell's cytoplasm, where they trigger a cascade of protein activation that leads to apoptosis. The NK cell binds to the abnormal target cell, releases its destructive payload, and detaches from the target cell. While the target cell undergoes apoptosis, the NK cell synthesizes more perforin and proteases to use on its next target.

NK cells contain these toxic compounds in granules in their cytoplasm. When stained, the granules are azurophilic and can be visualized under a light microscope ([\[link\]](#)). Even though they have granules, NK cells are not considered granulocytes because their granules are far less numerous than those found in true granulocytes. Furthermore, NK cells have a different lineage than granulocytes, arising from lymphoid rather than myeloid stem cells ([\[link\]](#)).

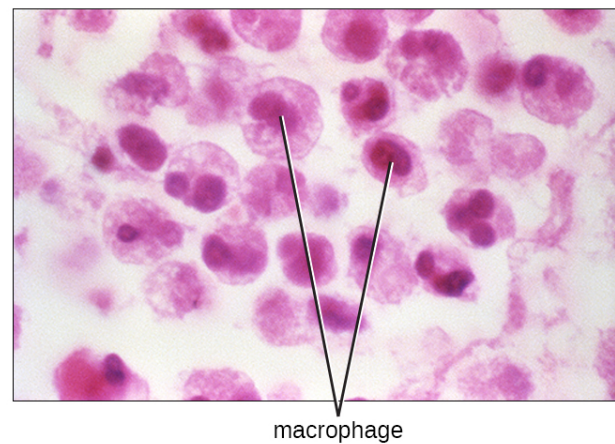
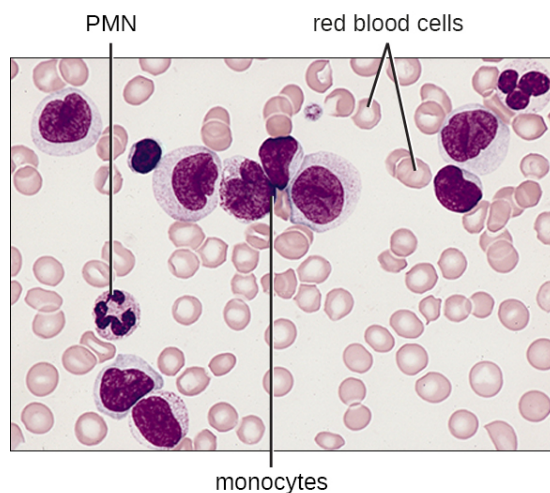


Natural killer cell with perforin-containing granules. (credit: modification of work by Rolstad B)

Monocytes

The largest of the white blood cells, **monocytes** have a nucleus that lacks lobes, and they also lack granules in the cytoplasm ([link](#)). Nevertheless, they are effective phagocytes, engulfing pathogens and apoptotic cells to help fight infection.

When monocytes leave the bloodstream and enter a specific body tissue, they differentiate into tissue-specific phagocytes called **macrophages** and **dendritic cells**. They are particularly important residents of lymphoid tissue, as well as nonlymphoid sites and organs. Macrophages and dendritic cells can reside in body tissues for significant lengths of time. Macrophages in specific body tissues develop characteristics suited to the particular tissue. Not only do they provide immune protection for the tissue in which they reside but they also support normal function of their neighboring tissue cells through the production of cytokines. Macrophages are given tissue-specific names, and a few examples of tissue-specific macrophages are listed in [\[link\]](#). Dendritic cells are important sentinels residing in the skin and mucous membranes, which are portals of entry for many pathogens. Monocytes, macrophages, and dendritic cells are all highly phagocytic and important promoters of the immune response through their production and release of cytokines. These cells provide an essential bridge between innate and adaptive immune responses, as discussed in the next section as well as the next chapter.



Monocytes are large, agranular white blood cells with a nucleus that lacks lobes. When monocytes leave the bloodstream, they differentiate and become macrophages with tissue-specific properties. (credit left: modification of work by Armed Forces Institute of Pathology; credit right: modification of work by Centers for Disease Control and Prevention)

Macrophages Found in Various Body Tissues	
Tissue	Macrophage
Brain and central nervous system	Microglial cells
Liver	Kupffer cells
Lungs	Alveolar macrophages (dust cells)
Peritoneal cavity	Peritoneal macrophages

Note:

- Describe the signals that activate natural killer cells.
- What is the difference between monocytes and macrophages?

Key Concepts and Summary

- The **formed elements** of the blood include red blood cells (**erythrocytes**), white blood cells (**leukocytes**), and **platelets**

(thrombocytes). Of these, leukocytes are primarily involved in the immune response.

- All formed elements originate in the bone marrow as stem cells (HSCs) that differentiate through **hematopoiesis**.
- **Granulocytes** are leukocytes characterized by a lobed nucleus and granules in the cytoplasm. These include **neutrophils (PMNs)**, **eosinophils**, and **basophils**.
- Neutrophils are the leukocytes found in the largest numbers in the bloodstream and they primarily fight bacterial infections.
- Eosinophils target parasitic infections. Eosinophils and basophils are involved in allergic reactions. Both release histamine and other proinflammatory compounds from their granules upon stimulation.
- **Mast cells** function similarly to basophils but can be found in tissues outside the bloodstream.
- **Natural killer (NK)** cells are lymphocytes that recognize and kill abnormal or infected cells by releasing proteins that trigger apoptosis.
- **Monocytes** are large, mononuclear leukocytes that circulate in the bloodstream. They may leave the bloodstream and take up residence in body tissues, where they differentiate and become tissue-specific **macrophages** and **dendritic cells**.

Multiple Choice

Exercise:

Problem:

White blood cells are also referred to as which of the following?

- A. platelets
- B. erythrocytes
- C. leukocytes
- D. megakaryocytes

Solution:

C

Exercise:

Problem: Hematopoiesis occurs in which of the following?

- A. liver
 - B. bone marrow
 - C. kidneys
 - D. central nervous system
-

Solution:

B

Exercise:

Problem: Granulocytes are which type of cell?

- A. lymphocyte
 - B. erythrocyte
 - C. megakaryocyte
 - D. leukocyte
-

Solution:

D

Matching**Exercise:**

Problem: Match each cell type with its description.

___natural killer cell	A. stains with basic dye methylene blue, has large amounts of histamine in granules, and facilitates allergic responses and inflammation
___basophil	B. stains with acidic dye eosin, has histamine and major basic protein in granules, and facilitates responses to protozoa and helminths
___macrophage	C. recognizes abnormal cells, binds to them, and releases perforin and granzyme molecules, which induce apoptosis
___eosinophil	D. large agranular phagocyte that resides in tissues such as the brain and lungs

Solution:

C, A, D, B

Exercise:

Problem:

Match each cellular defense with the infection it would most likely target.

___natural killer cell	A. virus-infected cell
___neutrophil	B. tapeworm in the intestines

____eosinophil

C. bacteria in a skin lesion

Solution:

A, C, B

Fill in the Blank

Exercise:

Problem: Platelets are also called _____.

Solution:

thrombocytes

Exercise:

Problem:

The cell in the bone marrow that gives rise to all other blood cell types is the _____.

Solution:

pluripotent hematopoietic stem cell (HSC)

Exercise:

Problem: PMNs are another name for _____.

Solution:

neutrophils

Exercise:

Problem: Kupffer cells residing in the liver are a type of _____.

Solution:

macrophage

Exercise:

Problem:

_____ are similar to basophils, but reside in tissues rather than circulating in the blood.

Solution:

mast cells

Short Answer

Exercise:

Problem:

Explain the difference between plasma and the formed elements of the blood.

Exercise:

Problem:

List three ways that a neutrophil can destroy an infectious bacterium.

Critical Thinking

Exercise:

Problem:

Neutrophils can sometimes kill human cells along with pathogens when they release the toxic contents of their granules into the surrounding tissue. Likewise, natural killer cells target human cells for destruction. Explain why it is advantageous for the immune system to have cells that can kill human cells as well as pathogens.

Exercise:**Problem:**

Refer to [\[link\]](#). In a blood smear taken from a healthy patient, which type of leukocyte would you expect to observe in the highest numbers?

LEARNING OBJECTIVES

- Explain how leukocytes migrate from peripheral blood into infected tissues
- Explain the mechanisms by which leukocytes recognize pathogens
- Explain the process of phagocytosis and the mechanisms by which phagocytes destroy and degrade pathogens

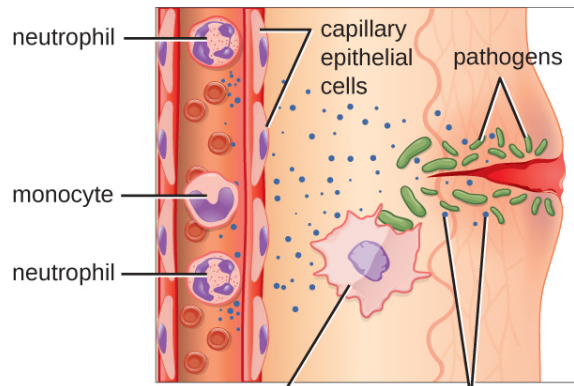
Several of the cell types discussed in the previous section can be described as phagocytes—cells whose main function is to seek, ingest, and kill pathogens. This process, called phagocytosis, was first observed in starfish in the 1880s by Nobel Prize-winning zoologist Ilya Metchnikoff (1845–1916), who made the connection to white blood cells (WBCs) in humans and other animals. At the time, Pasteur and other scientists believed that WBCs were spreading pathogens rather than killing them (which is true for some diseases, such as tuberculosis). But in most cases, phagocytes provide a strong, swift, and effective defense against a broad range of microbes, making them a critical component of innate nonspecific immunity. This section will focus on the mechanisms by which phagocytes are able to seek, recognize, and destroy pathogens.

Extravasation (Diapedesis) of Leukocytes

Some phagocytes are leukocytes (WBCs) that normally circulate in the bloodstream. To reach pathogens located in infected tissue, leukocytes must pass through the walls of small capillary blood vessels within tissues. This

process, called **extravasation**, or **diapedesis**, is initiated by complement factor C5a, as well as cytokines released into the immediate vicinity by resident macrophages and tissue cells responding to the presence of the infectious agent ([\[link\]](#)). Similar to C5a, many of these cytokines are proinflammatory and chemotactic, and they bind to cells of small capillary blood vessels, initiating a response in the endothelial cells lining the inside of the blood vessel walls. This response involves the upregulation and expression of various cellular adhesion molecules and receptors. Leukocytes passing through will stick slightly to the adhesion molecules, slowing down and rolling along the blood vessel walls near the infected area. When they reach a cellular junction, they will bind to even more of these adhesion molecules, flattening out and squeezing through the cellular junction in a process known as **transendothelial migration**. This mechanism of “rolling adhesion” allows leukocytes to exit the bloodstream and enter the infected areas, where they can begin phagocytosing the invading pathogens.

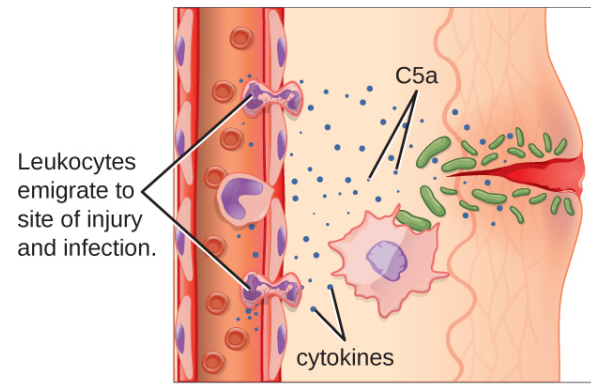
Note that extravasation does not occur in arteries or veins. These blood vessels are surrounded by thicker, multilayer protective walls, in contrast to the thin single-cell-layer walls of capillaries. Furthermore, the blood flow in arteries is too turbulent to allow for rolling adhesion. Also, some leukocytes tend to respond to an infection more quickly than others. The first to arrive typically are neutrophils, often within hours of a bacterial infection. By contrast, monocytes may take several days to leave the bloodstream and differentiate into macrophages.



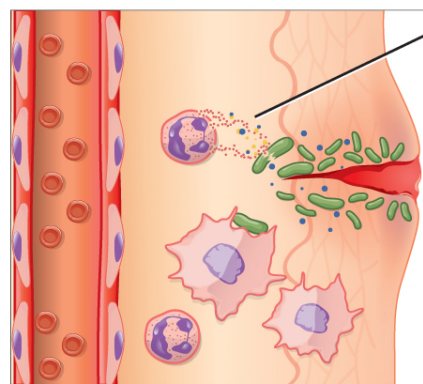
Resident macrophage engulfs pathogen and releases proinflammatory, chemotactic cytokines.

Injured/infected cells secrete chemical signals into the blood.

- 1 Leukocytes in the blood respond to chemical attractants released by pathogens and chemical signals from nearby injured cells.



- 2 The leukocytes squeeze between the cells of the capillary wall as they follow the chemical signals to where they are most concentrated (positive chemotaxis).



Neutrophil releases cytotoxic chemicals from granules into tissue.

- 3 Within the damaged tissue, neutrophils release chemicals that break apart pathogens. Monocytes differentiate into macrophages. Neutrophils and macrophages phagocytize pathogens and cellular debris.

Damaged cells and macrophages that have ingested pathogens release cytokines that are proinflammatory and chemotactic for leukocytes. In addition, activation of complement at the site of infection results in production of the chemotactic and proinflammatory C5a. Leukocytes exit the blood vessel and follow the chemoattractant signal of cytokines and C5a to the site of infection. Granulocytes such as neutrophils release chemicals that destroy pathogens. They are also capable of phagocytosis and intracellular killing of bacterial pathogens.

Note:

Watch the following videos on [leukocyte extravasation](#) and [leukocyte rolling](#) to learn more.

Note:

- Explain the role of adhesion molecules in the process of extravasation.

Pathogen Recognition

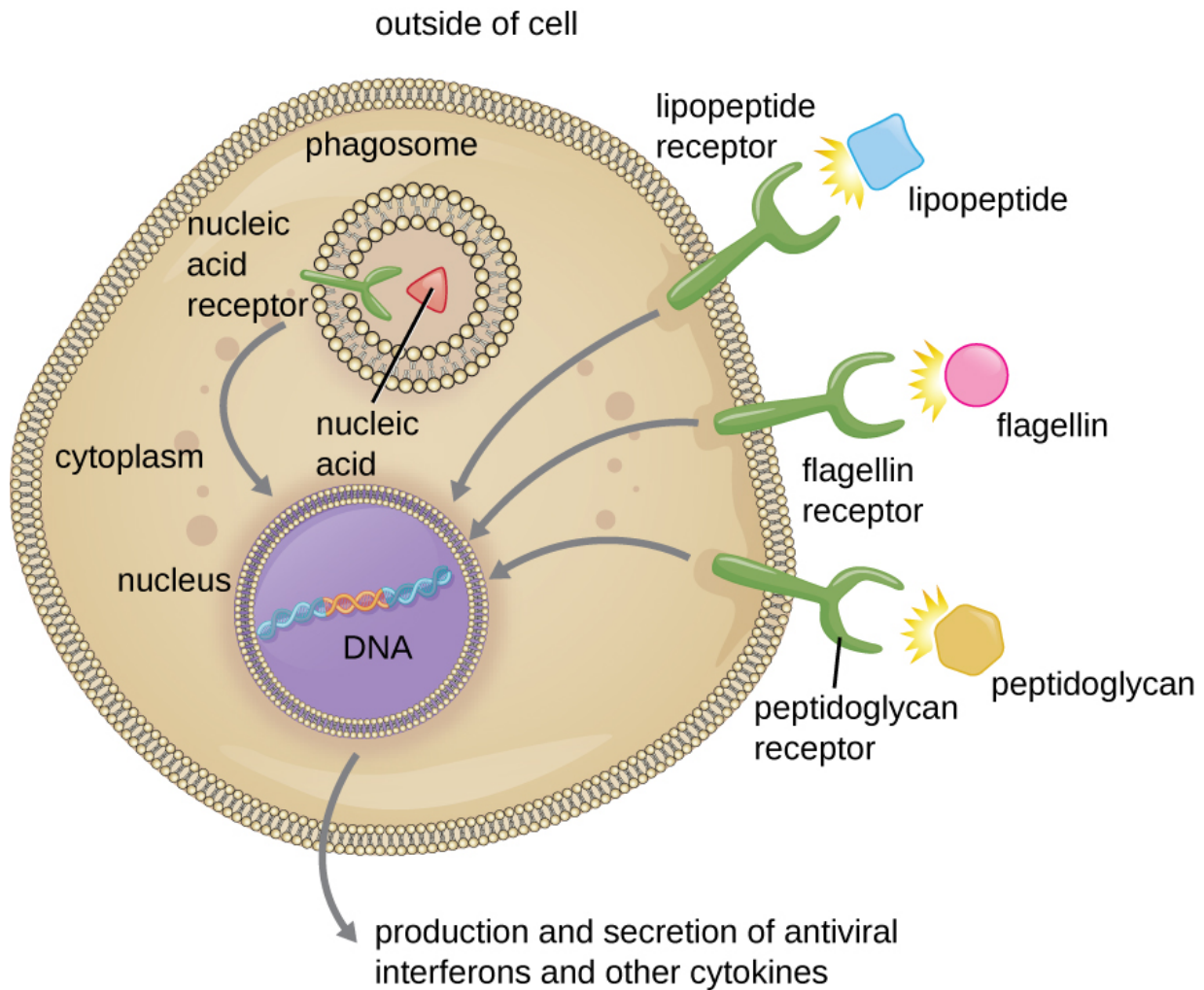
As described in the previous section, opsonization of pathogens by antibody; complement factors C1q, C3b, and C4b; and lectins can assist phagocytic cells in recognition of pathogens and attachment to initiate phagocytosis. However, not all pathogen recognition is opsonin dependent. Phagocytes can also recognize molecular structures that are common to many groups of pathogenic microbes. Such structures are called **pathogen-associated molecular patterns (PAMPs)**. Common PAMPs include the following:

- peptidoglycan, found in bacterial cell walls;
- flagellin, a protein found in bacterial flagella;
- lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria;
- lipopeptides, molecules expressed by most bacteria; and
- nucleic acids such as viral DNA or RNA.

Like numerous other PAMPs, these substances are integral to the structure of broad classes of microbes.

The structures that allow phagocytic cells to detect PAMPs are called **pattern recognition receptors (PRRs)**. One group of PRRs is the **toll-like receptors (TLRs)**, which bind to various PAMPs and communicate with the nucleus of the phagocyte to elicit a response. Many TLRs (and other PRRs) are located on the surface of a phagocyte, but some can also be found embedded in the membranes of interior compartments and organelles ([link](#)). These interior PRRs can be useful for the binding and recognition of intracellular pathogens that may have gained access to the inside of the cell before phagocytosis could take place. Viral nucleic acids, for example, might encounter an interior PRR, triggering production of the antiviral cytokine interferon.

In addition to providing the first step of pathogen recognition, the interaction between PAMPs and PRRs on macrophages provides an intracellular signal that activates the phagocyte, causing it to transition from a dormant state of readiness and slow proliferation to a state of hyperactivity, proliferation, production/secretion of cytokines, and enhanced intracellular killing. PRRs on macrophages also respond to chemical distress signals from damaged or stressed cells. This allows macrophages to extend their responses beyond protection from infectious diseases to a broader role in the inflammatory response initiated from injuries or other diseases.



Phagocytic cells contain pattern recognition receptors (PRRs) capable of recognizing various pathogen-associated molecular patterns (PAMPs). These PRRs can be found on the plasma membrane or in internal phagosomes. When a PRR recognizes a PAMP, it sends a signal to the nucleus that activates genes involved in phagocytosis, cellular proliferation, production and secretion of antiviral interferons and proinflammatory cytokines, and enhanced intracellular killing.

Note:

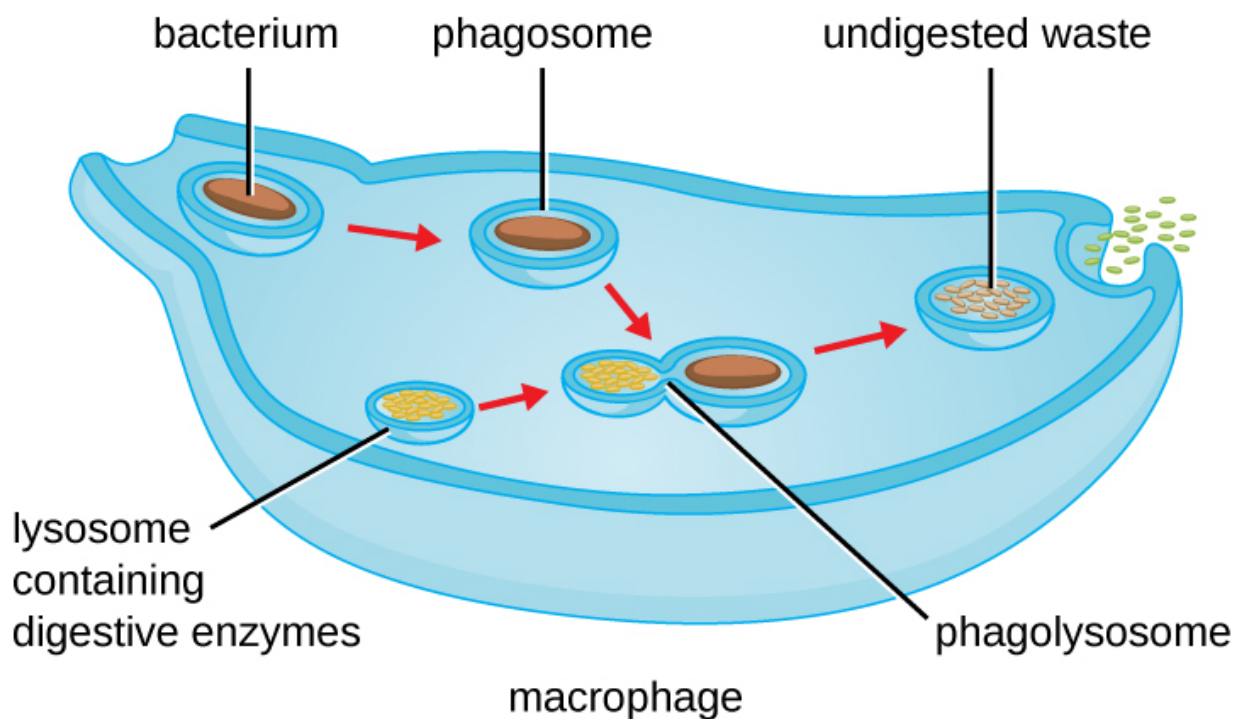
- Name four pathogen-associated molecular patterns (PAMPs).
- Describe the process of phagocyte activation.

Pathogen Degradation

Once pathogen recognition and attachment occurs, the pathogen is engulfed in a vesicle and brought into the internal compartment of the phagocyte in a process called **phagocytosis** ([link](#)). PRRs can aid in phagocytosis by first binding to the pathogen's surface, but phagocytes are also capable of engulfing nearby items even if they are not bound to specific receptors. To engulf the pathogen, the phagocyte forms a pseudopod that wraps around the pathogen and then pinches it off into a membrane vesicle called a **phagosome**. Acidification of the phagosome (pH decreases to the range of 4–5) provides an important early antibacterial mechanism. The phagosome containing the pathogen fuses with one or more lysosomes, forming a **phagolysosome**. Formation of the phagolysosome enhances the acidification, which is essential for activation of pH-dependent digestive lysosomal enzymes and production of hydrogen peroxide and toxic reactive oxygen species. Lysosomal enzymes such as lysozyme, phospholipase, and proteases digest the pathogen. Other enzymes are involved in a respiratory burst. During the respiratory burst, phagocytes will increase their uptake and consumption of oxygen, but not for energy production. The increased oxygen consumption is focused on the production of superoxide anion, hydrogen peroxide, hydroxyl radicals, and other reactive oxygen species that are antibacterial.

In addition to the reactive oxygen species produced by the respiratory burst, reactive nitrogen compounds with cytotoxic (cell-killing) potential can also form. For example, nitric oxide can react with superoxide to form peroxynitrite, a highly reactive nitrogen compound with degrading capabilities similar to those of the reactive oxygen species. Some phagocytes even contain an internal storehouse of microbicidal defensin proteins (e.g., neutrophil granules). These destructive forces can be released into the area around the cell to degrade microbes externally. Neutrophils, especially, can be quite efficient at this secondary antimicrobial mechanism.

Once degradation is complete, leftover waste products are excreted from the cell in an exocytic vesicle. However, it is important to note that not all remains of the pathogen are excreted as waste. Macrophages and dendritic cells are also antigen-presenting cells involved in the specific adaptive immune response. These cells further process the remains of the degraded pathogen and present key antigens (specific pathogen proteins) on their cellular surface. This is an important step for stimulation of some adaptive immune responses, as will be discussed in more detail in the next chapter.



The stages of phagocytosis include the engulfment of a pathogen, the formation of a phagosome, the digestion of the pathogenic particle in the phagolysosome, and the expulsion of undigested materials from the cell.

Note:

Visit this [link](#) to view a phagocyte chasing and engulfing a pathogen.

Note:

- What is the difference between a phagosome and a lysosome?

Note:

When Phagocytosis Fails

Although phagocytosis successfully destroys many pathogens, some are able to survive and even exploit this defense mechanism to multiply in the body and cause widespread infection. Protozoans of the genus *Leishmania* are one example. These obligate intracellular parasites are flagellates transmitted to humans by the bite of a sand fly. Infections cause serious and sometimes disfiguring sores and ulcers in the skin and other tissues ([\[link\]](#)). Worldwide, an estimated 1.3 million people are newly infected with leishmaniasis annually.[\[footnote\]](#)

World Health Organization. “Leishmaniasis.” 2016.

<http://www.who.int/mediacentre/factsheets/fs375/en/>.

Salivary peptides from the sand fly activate host macrophages at the site of their bite. The classic or alternate pathway for complement activation ensues with C3b opsonization of the parasite. *Leishmania* cells are phagocytosed, lose their flagella, and multiply in a form known as an amastigote (Leishman-Donovan body) within the phagolysosome.

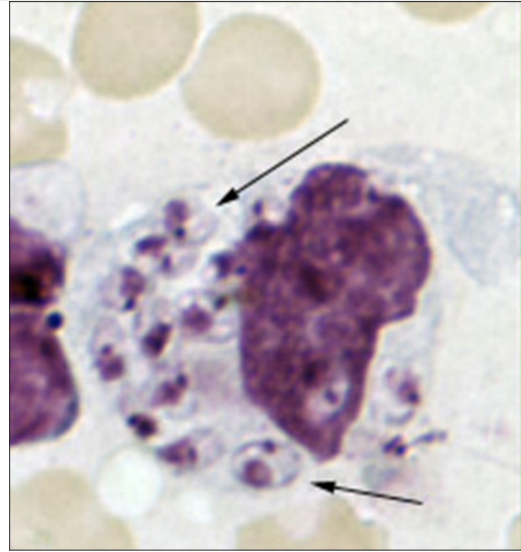
Although many other pathogens are destroyed in the phagolysosome, survival of the *Leishmania* amastigotes is maintained by the presence of surface lipophosphoglycan and acid phosphatase. These substances inhibit the macrophage respiratory burst and lysosomal enzymes. The parasite then multiplies inside the cell and lyses the infected macrophage, releasing the amastigotes to infect other macrophages within the same host. Should another sand fly bite an infected person, it might ingest amastigotes and then transmit them to another individual through another bite.

There are several different forms of leishmaniasis. The most common is a localized cutaneous form of the illness caused by *L. tropica*, which

typically resolves spontaneously over time but with some significant lymphocyte infiltration and permanent scarring. A mucocutaneous form of the disease, caused by *L. viannia brasiliensis*, produces lesions in the tissue of the nose and mouth and can be life threatening. A visceral form of the illness can be caused by several of the different *Leishmania* species. It affects various organ systems and causes abnormal enlargement of the liver and spleen. Irregular fevers, anemia, liver dysfunction, and weight loss are all signs and symptoms of visceral leishmaniasis. If left untreated, it is typically fatal.



(a)



(b)

(a) Cutaneous leishmaniasis is a disfiguring disease caused by the intracellular flagellate *Leishmania tropica*, transmitted by the bite of a sand fly. (b) This light micrograph of a sample taken from a skin lesion shows a large cell, which is a macrophage infected with *L. tropica* amastigotes (arrows). The amastigotes have lost their flagella but their nuclei are visible. Soon the amastigotes will lyse the macrophage and be engulfed by other phagocytes, spreading the infection. (credit a: modification of work by Otis Historical Archives of “National Museum of Health & Medicine”; credit b: modification of work by Centers for Disease Control and Prevention)

Key Concepts and Summary

- Phagocytes are cells that recognize pathogens and destroy them through phagocytosis.
- Recognition often takes place by the use of phagocyte receptors that bind molecules commonly found on pathogens, known as **pathogen-associated molecular patterns (PAMPs)**.
- The receptors that bind PAMPs are called **pattern recognition receptors**, or **PRRs**. **Toll-like receptors (TLRs)** are one type of PRR found on phagocytes.
- **Extravasation** of white blood cells from the bloodstream into infected tissue occurs through the process of **transendothelial migration**.
- Phagocytes degrade pathogens through **phagocytosis**, which involves engulfing the pathogen, killing and digesting it within a **phagolysosome**, and then excreting undigested matter.

Multiple Choice

Exercise:

Problem:

PAMPs would be found on the surface of which of the following?

- A. pathogen
- B. phagocyte
- C. skin cell
- D. blood vessel wall

Solution:

A

Exercise:

Problem:

_____ on phagocytes bind to PAMPs on bacteria, which triggers the uptake and destruction of the bacterial pathogens?

- A. PRRs
- B. AMPs
- C. PAMPs
- D. PMNs

Solution:

A

Exercise:**Problem:**

Which of the following best characterizes the mode of pathogen recognition for opsonin-dependent phagocytosis?

- A. Opsonins produced by a pathogen attract phagocytes through chemotaxis.
- B. A PAMP on the pathogen's surface is recognized by a phagocyte's toll-like receptors.
- C. A pathogen is first coated with a molecule such as a complement protein, which allows it to be recognized by phagocytes.
- D. A pathogen is coated with a molecule such as a complement protein that immediately lyses the cell.

Solution:

C

Fill in the Blank

Exercise:**Problem:**

_____, also known as diapedesis, refers to the exit from the bloodstream of neutrophils and other circulating leukocytes.

Solution:

extravasation

Exercise:

Problem: Toll-like receptors are examples of _____.

Solution:

pattern-recognition receptors (PRRs)

Short Answer**Exercise:****Problem:**

Briefly summarize the events leading up to and including the process of transendothelial migration.

Inflammation and Fever

LEARNING OBJECTIVES

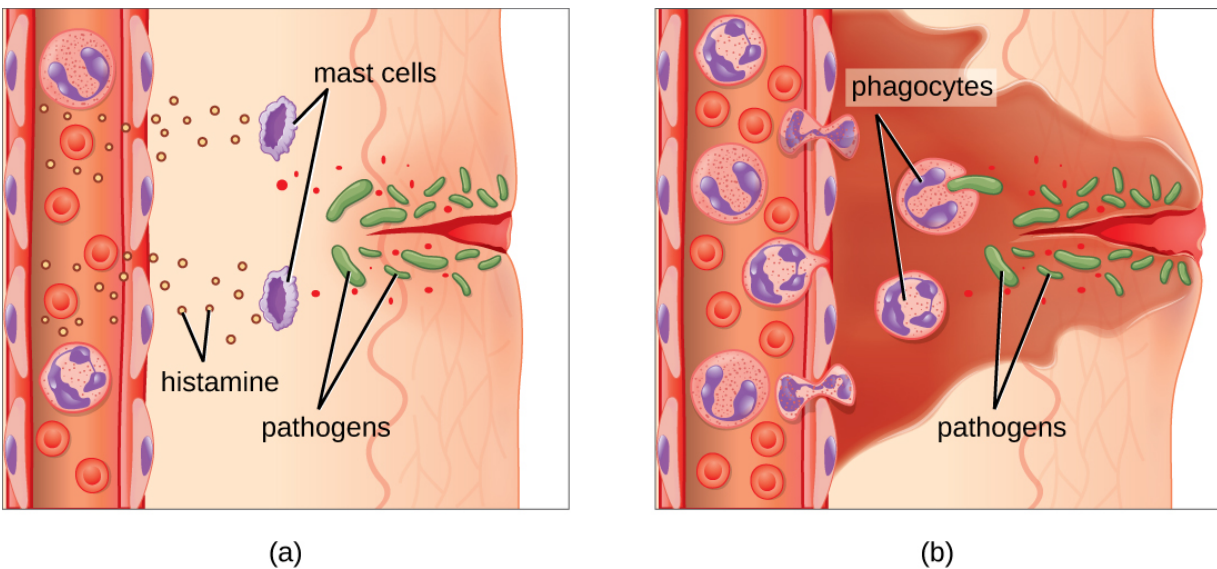
- Identify the signs of inflammation and fever and explain why they occur
- Explain the advantages and risks posed by inflammatory responses

The inflammatory response, or **inflammation**, is triggered by a cascade of chemical mediators and cellular responses that may occur when cells are damaged and stressed or when pathogens successfully breach the physical barriers of the innate immune system. Although inflammation is typically associated with negative consequences of injury or disease, it is a necessary process insofar as it allows for recruitment of the cellular defenses needed to eliminate pathogens, remove damaged and dead cells, and initiate repair mechanisms. Excessive inflammation, however, can result in local tissue damage and, in severe cases, may even become deadly.

Acute Inflammation

An early, if not immediate, response to tissue injury is acute inflammation. Immediately following an injury, vasoconstriction of blood vessels will occur to minimize blood loss. The amount of vasoconstriction is related to the amount of vascular injury, but it is usually brief. Vasoconstriction is followed by vasodilation and increased vascular permeability, as a direct result of the release of histamine from resident mast cells. Increased blood flow and vascular permeability can dilute toxins and bacterial products at the site of injury or infection. They also contribute to the five observable

signs associated with the inflammatory response: **erythema** (redness), **edema** (swelling), heat, pain, and altered function. Vasodilation and increased vascular permeability are also associated with an influx of phagocytes at the site of injury and/or infection. This can enhance the inflammatory response because phagocytes may release proinflammatory chemicals when they are activated by cellular distress signals released from damaged cells, by PAMPs, or by opsonins on the surface of pathogens. Activation of the complement system can further enhance the inflammatory response through the production of the anaphylatoxin C5a. [\[link\]](#) illustrates a typical case of acute inflammation at the site of a skin wound.



(a) Mast cells detect injury to nearby cells and release histamine, initiating an inflammatory response. (b) Histamine increases blood flow to the wound site, and increased vascular permeability allows fluid, proteins, phagocytes, and other immune cells to enter infected tissue. These events result in the swelling and reddening of the injured site, and the increased blood flow to the injured site causes it to feel warm. Inflammation is also associated with pain due to these events stimulating nerve pain receptors in the tissue. The interaction of phagocyte PRRs with cellular distress signals and PAMPs and opsonins on the surface of pathogens leads to the release of more proinflammatory chemicals, enhancing the inflammatory response.

During the period of inflammation, the release of bradykinin causes capillaries to remain dilated, flooding tissues with fluids and leading to edema. Increasing numbers of neutrophils are recruited to the area to fight pathogens. As the fight rages on, pus forms from the accumulation of neutrophils, dead cells, tissue fluids, and lymph. Typically, after a few days, macrophages will help to clear out this pus. Eventually, tissue repair can begin in the wounded area.

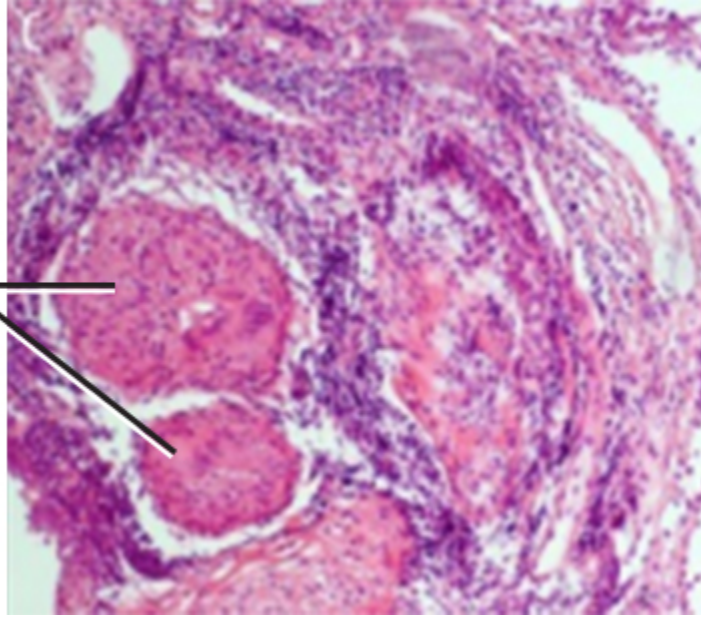
Chronic Inflammation

When acute inflammation is unable to clear an infectious pathogen, chronic inflammation may occur. This often results in an ongoing (and sometimes futile) lower-level battle between the host organism and the pathogen. The wounded area may heal at a superficial level, but pathogens may still be present in deeper tissues, stimulating ongoing inflammation. Additionally, chronic inflammation may be involved in the progression of degenerative neurological diseases such as Alzheimer's and Parkinson's, heart disease, and metastatic cancer.

Chronic inflammation may lead to the formation of **granulomas**, pockets of infected tissue walled off and surrounded by WBCs. Macrophages and other phagocytes wage an unsuccessful battle to eliminate the pathogens and dead cellular materials within a granuloma. One example of a disease that produces chronic inflammation is tuberculosis, which results in the formation of granulomas in lung tissues. A tubercular granuloma is called a tubercle ([\[link\]](#)). Tuberculosis will be covered in more detail in [Bacterial Infections of the Respiratory Tract](#).

Chronic inflammation is not just associated with bacterial infections. Chronic inflammation can be an important cause of tissue damage from viral infections. The extensive scarring observed with hepatitis C infections and liver cirrhosis is the result of chronic inflammation.

granuloma



A tubercle is a granuloma in the lung tissue of a patient with tuberculosis. In this micrograph, white blood cells (stained purple) have walled off a pocket of tissue infected with *Mycobacterium tuberculosis*. Granulomas also occur in many other forms of disease. (credit: modification of work by Piotrowski WJ, Górski P, Duda-Szymańska J, Kwiatkowska S)

Note:

- Name the five signs of inflammation.
- Is a granuloma an acute or chronic form of inflammation? Explain.

Note:

Chronic Edema

In addition to granulomas, chronic inflammation can also result in long-term edema. A condition known as lymphatic filariasis (also known as elephantiasis) provides an extreme example. Lymphatic filariasis is caused by microscopic nematodes (parasitic worms) whose larvae are transmitted between human hosts by mosquitoes. Adult worms live in the lymphatic vessels, where their presence stimulates infiltration by lymphocytes, plasma cells, eosinophils, and thrombocytes (a condition known as lymphangitis). Because of the chronic nature of the illness, granulomas, fibrosis, and blocking of the lymphatic system may eventually occur. Over time, these blockages may worsen with repeated infections over decades, leading to skin thickened with edema and fibrosis. Lymph (extracellular tissue fluid) may spill out of the lymphatic areas and back into tissues, causing extreme swelling ([\[link\]](#)). Secondary bacterial infections commonly follow. Because it is a disease caused by a parasite, eosinophilia (a dramatic rise in the number of eosinophils in the blood) is characteristic of acute infection. However, this increase in antiparasite granulocytes is not sufficient to clear the infection in many cases.

Lymphatic filariasis affects an estimated 120 million people worldwide, mostly concentrated in Africa and Asia.[\[footnote\]](#) Improved sanitation and mosquito control can reduce transmission rates.

Centers for Disease Control and Prevention. “Parasites–Lymphatic Filariasis.” 2016.

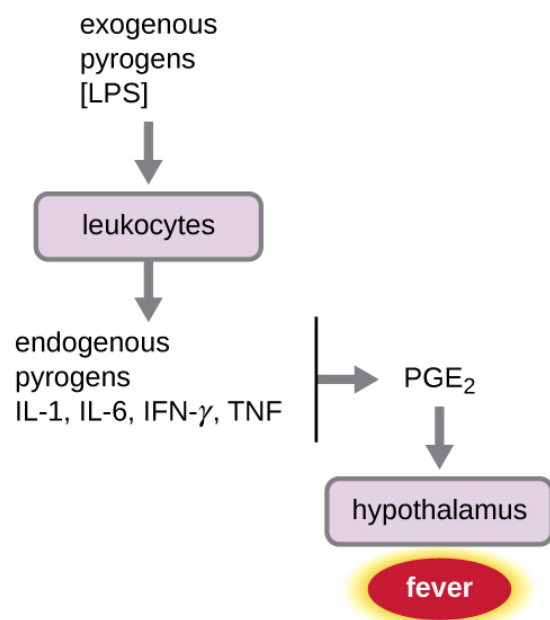
http://www.cdc.gov/parasites/lymphaticfilariasis/gen_info/faqs.html.



Elephantiasis (chronic edema) of the legs due to filariasis. (credit: modification of work by Centers for Disease Control and Prevention)

Fever

A **fever** is an inflammatory response that extends beyond the site of infection and affects the entire body, resulting in an overall increase in body temperature. Body temperature is normally regulated and maintained by the hypothalamus, an anatomical section of the brain that functions to maintain homeostasis in the body. However, certain bacterial or viral infections can result in the production of **pyrogens**, chemicals that effectively alter the “thermostat setting” of the hypothalamus to elevate body temperature and cause fever. Pyrogens may be exogenous or endogenous. For example, the endotoxin lipopolysaccharide (LPS), produced by gram-negative bacteria, is an exogenous pyrogen that may induce the leukocytes to release endogenous pyrogens such as interleukin-1 (IL-1), IL-6, interferon- γ (IFN- γ), and tumor necrosis factor (TNF). In a cascading effect, these molecules can then lead to the release of prostaglandin E2 (PGE₂) from other cells, resetting the hypothalamus to initiate fever ([\[link\]](#)).



The role of the hypothalamus in the inflammatory response. Macrophages recognize pathogens in an area and release cytokines that trigger inflammation. The cytokines also send a signal up the vagus nerve to the hypothalamus.

Like other forms of inflammation, a fever enhances the innate immune defenses by stimulating leukocytes to kill pathogens. The rise in body temperature also may inhibit the growth of many pathogens since human pathogens are mesophiles with optimum growth occurring around 35 °C (95 °F). In addition, some studies suggest that fever may also stimulate release of iron-sequestering compounds from the liver, thereby starving out microbes that rely on iron for growth.[\[footnote\]](#)

N. Parrow et al. “Sequestration and Scavenging of Iron in Infection.” *Infection and Immunity* 81 no. 10 (2013):3503–3514

During fever, the skin may appear pale due to vasoconstriction of the blood vessels in the skin, which is mediated by the hypothalamus to divert blood flow away from extremities, minimizing the loss of heat and raising the core temperature. The hypothalamus will also stimulate shivering of muscles, another effective mechanism of generating heat and raising the core temperature.

The **crisis phase** occurs when the fever breaks. The hypothalamus stimulates vasodilation, resulting in a return of blood flow to the skin and a subsequent release of heat from the body. The hypothalamus also stimulates sweating, which cools the skin as the sweat evaporates.

Although a low-level fever may help an individual overcome an illness, in some instances, this immune response can be too strong, causing tissue and organ damage and, in severe cases, even death. The inflammatory response to bacterial superantigens is one scenario in which a life-threatening fever may develop. Superantigens are bacterial or viral proteins that can cause an excessive activation of T cells from the specific adaptive immune defense, as well as an excessive release of cytokines that overstimulates the inflammatory response. For example, *Staphylococcus aureus* and *Streptococcus pyogenes* are capable of producing superantigens that cause toxic shock syndrome and scarlet fever, respectively. Both of these conditions can be associated with very high, life-threatening fevers in excess of 42 °C (108 °F).

Note:

- Explain the difference between exogenous and endogenous pyrogens.
- How does a fever inhibit pathogens?

Note:**Resolution**

Given her father's premature death, Angela's doctor suspects that she has hereditary angioedema, a genetic disorder that compromises the function of C1 inhibitor protein. Patients with this genetic abnormality may have occasional episodes of swelling in various parts of the body. In Angela's case, the swelling has occurred in the respiratory tract, leading to difficulty breathing. Swelling may also occur in the gastrointestinal tract, causing abdominal cramping, diarrhea, and vomiting, or in the muscles of the face or limbs. This swelling may be nonresponsive to steroid treatment and is often misdiagnosed as an allergy.

Because there are three types of hereditary angioedema, the doctor orders a more specific blood test to look for levels of C1-INH, as well as a functional assay of Angela's C1 inhibitors. The results suggest that Angela has type I hereditary angioedema, which accounts for 80%–85% of all cases. This form of the disorder is caused by a deficiency in C1 esterase inhibitors, the proteins that normally help suppress activation of the complement system. When these proteins are deficient or nonfunctional, overstimulation of the system can lead to production of inflammatory anaphylatoxins, which results in swelling and fluid buildup in tissues. There is no cure for hereditary angioedema, but timely treatment with purified and concentrated C1-INH from blood donors can be effective, preventing tragic outcomes like the one suffered by Angela's father. A number of therapeutic drugs, either currently approved or in late-stage human trials, may also be considered as options for treatment in the near future. These drugs work by inhibiting inflammatory molecules or the receptors for inflammatory molecules.

Thankfully, Angela's condition was quickly diagnosed and treated. Although she may experience additional episodes in the future, her prognosis is good and she can expect to live a relatively normal life provided she seeks treatment at the onset of symptoms.

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- **Inflammation** results from the collective response of chemical mediators and cellular defenses to an injury or infection.
- **Acute inflammation** is short lived and localized to the site of injury or infection. **Chronic inflammation** occurs when the inflammatory response is unsuccessful, and may result in the formation of **granulomas** (e.g., with tuberculosis) and scarring (e.g., with hepatitis C viral infections and liver cirrhosis).
- The five cardinal signs of inflammation are **erythema**, **edema**, heat, pain, and altered function. These largely result from innate responses that draw increased blood flow to the injured or infected tissue.
- **Fever** is a system-wide sign of inflammation that raises the body temperature and stimulates the immune response.
- Both inflammation and fever can be harmful if the inflammatory response is too severe.

Multiple Choice

Exercise:

Problem: Which refers to swelling as a result of inflammation?

- A. erythema
- B. edema
- C. granuloma
- D. vasodilation

Solution:

B

Exercise:

Problem:

Which type of inflammation occurs at the site of an injury or infection?

- A. acute
- B. chronic
- C. endogenous
- D. exogenous

Solution:

A

Fill in the Blank**Exercise:****Problem:**

A(n) _____ is a walled-off area of infected tissue that exhibits chronic inflammation.

Solution:

granuloma

Exercise:**Problem:**

The _____ is the part of the body responsible for regulating body temperature.

Solution:

hypothalamus

Exercise:**Problem:**

Heat and redness, or _____, occur when the small blood vessels in an inflamed area dilate (open up), bringing more blood much closer to the surface of the skin.

Solution:

erythema

Short Answer**Exercise:****Problem:**

Differentiate exogenous and endogenous pyrogens, and provide an example of each.

Critical Thinking**Exercise:****Problem:**

If a gram-negative bacterial infection reaches the bloodstream, large quantities of LPS can be released into the blood, resulting in a syndrome called septic shock. Death due to septic shock is a real danger. The overwhelming immune and inflammatory responses that occur with septic shock can cause a perilous drop in blood pressure; intravascular blood clotting; development of thrombi and emboli that block blood vessels, leading to tissue death; failure of multiple organs; and death of the patient. Identify and characterize two to three therapies that might be useful in stopping the dangerous events and outcomes of septic shock once it has begun, given what you have learned about inflammation and innate immunity in this chapter.

Exercise:**Problem:**

In Lubeck, Germany, in 1930, a group of 251 infants was accidentally administered a tainted vaccine for tuberculosis that contained live *Mycobacterium tuberculosis*. This vaccine was administered orally, directly exposing the infants to the deadly bacterium. Many of these infants contracted tuberculosis, and some died. However, 44 of the infants never contracted tuberculosis. Based on your knowledge of the innate immune system, what innate defenses might have inhibited *M. tuberculosis* enough to prevent these infants from contracting the disease?

Introduction

class="introduction"

Polio was once a common disease with potentially serious consequences, including paralysis. Vaccination has all but eliminated the disease from most countries around the world. An iron-lung ward, such as the one shown in this 1953 photograph, housed patients paralyzed from polio and unable to breathe for themselves.



People living in developed nations and born in the 1960s or later may have difficulty understanding the once heavy burden of devastating infectious diseases. For example, smallpox, a deadly viral disease, once destroyed entire civilizations but has since been eradicated. Thanks to the vaccination efforts by multiple groups, including the World Health Organization, Rotary International, and the United Nations Children's Fund (UNICEF), smallpox has not been diagnosed in a patient since 1977. Polio is another excellent example. This crippling viral disease paralyzed patients, who were often kept alive in "iron lung wards" as recently as the 1950s ([\[link\]](#)). Today, vaccination against polio has nearly eradicated the disease. Vaccines have also reduced the prevalence of once-common infectious diseases such as chickenpox, German measles, measles, mumps, and whooping cough. The success of these and other vaccines is due to the very specific and adaptive host defenses that are the focus of this chapter.

[Innate Nonspecific Host Defenses](#) described innate immunity against microbial pathogens. Higher animals, such as humans, also possess an adaptive immune defense, which is highly specific for individual microbial pathogens. This specific adaptive immunity is acquired through active infection or vaccination and serves as an important defense against pathogens that evade the defenses of innate immunity.

Overview of Specific Adaptive Immunity

LEARNING OBJECTIVES

- Define memory, primary response, secondary response, and specificity
- Distinguish between humoral and cellular immunity
- Differentiate between antigens, epitopes, and haptens
- Describe the structure and function of antibodies and distinguish between the different classes of antibodies

Note:

Part 1

Olivia, a one-year old infant, is brought to the emergency room by her parents, who report her symptoms: excessive crying, irritability, sensitivity to light, unusual lethargy, and vomiting. A physician feels swollen lymph nodes in Olivia's throat and armpits. In addition, the area of the abdomen over the spleen is swollen and tender.

- What do these symptoms suggest?
- What tests might be ordered to try to diagnose the problem?

Jump to the [next](#) Clinical Focus box.

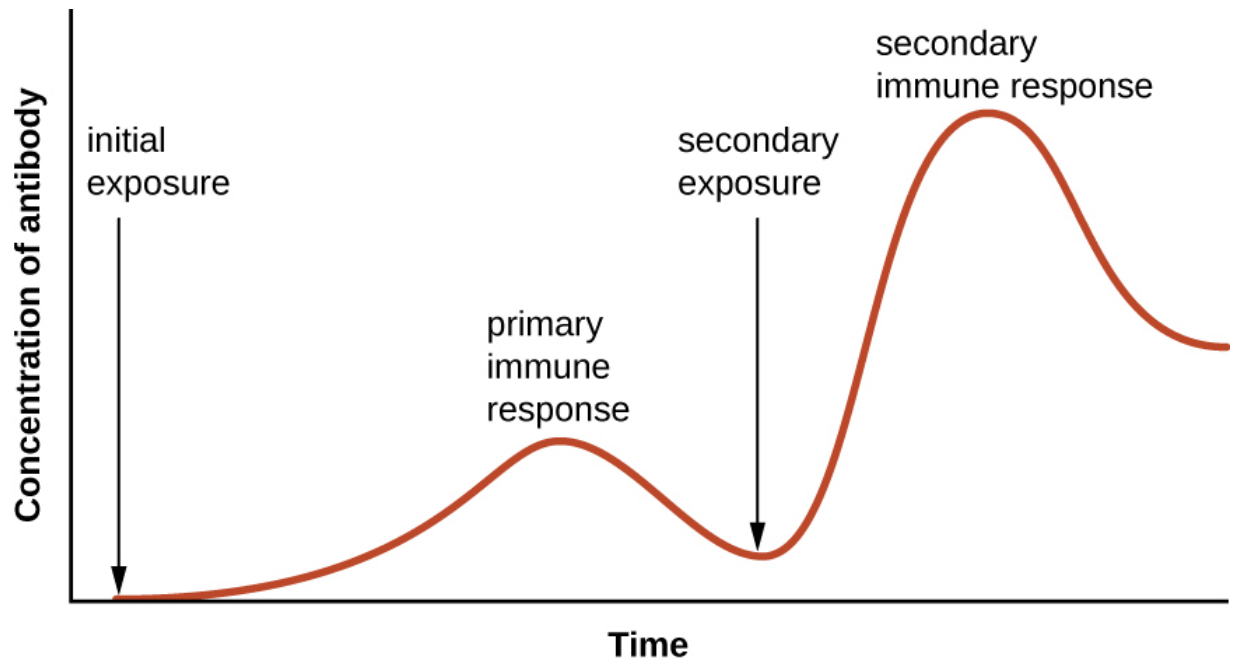
Adaptive immunity is defined by two important characteristics: **specificity** and **memory**. Specificity refers to the adaptive immune system's ability to target specific pathogens, and memory refers to its ability to quickly

respond to pathogens to which it has previously been exposed. For example, when an individual recovers from chickenpox, the body develops a *memory* of the infection that will *specifically* protect it from the causative agent, the varicella-zoster virus, if it is exposed to the virus again later.

Specificity and memory are achieved by essentially programming certain cells involved in the immune response to respond rapidly to subsequent exposures of the pathogen. This programming occurs as a result of the first exposure to a pathogen or vaccine, which triggers a **primary response**. Subsequent exposures result in a **secondary response** that is faster and stronger as a result of the body's memory of the first exposure ([\[link\]](#)). This secondary response, however, is specific to the pathogen in question. For example, exposure to one virus (e.g., varicella-zoster virus) will not provide protection against other viral diseases (e.g., measles, mumps, or polio).

Adaptive specific immunity involves the actions of two distinct cell types: **B lymphocytes (B cells)** and **T lymphocytes (T cells)**. Although B cells and T cells arise from a common hematopoietic stem cell differentiation pathway (see [\[link\]](#)), their sites of maturation and their roles in adaptive immunity are very different.

B cells mature in the bone marrow and are responsible for the production of glycoproteins called **antibodies**, or **immunoglobulins**. Antibodies are involved in the body's defense against pathogens and toxins in the extracellular environment. Mechanisms of adaptive specific immunity that involve B cells and antibody production are referred to as **humoral immunity**. The maturation of T cells occurs in the thymus. T cells function as the central orchestrator of both innate and adaptive immune responses. They are also responsible for destruction of cells infected with intracellular pathogens. The targeting and destruction of intracellular pathogens by T cells is called cell-mediated immunity, or **cellular immunity**.



This graph illustrates the primary and secondary immune responses related to antibody production after an initial and secondary exposure to an antigen. Notice that the secondary response is faster and provides a much higher concentration of antibody.

Note:

- List the two defining characteristics of adaptive immunity.
- Explain the difference between a primary and secondary immune response.
- How do humoral and cellular immunity differ?

Antigens

Activation of the adaptive immune defenses is triggered by pathogen-specific molecular structures called **antigens**. Antigens are similar to the pathogen-associated molecular patterns (PAMPs) discussed in [Pathogen Recognition and Phagocytosis](#); however, whereas PAMPs are molecular structures found on numerous pathogens, antigens are unique to a specific pathogen. The antigens that stimulate adaptive immunity to chickenpox, for example, are unique to the varicella-zoster virus but significantly different from the antigens associated with other viral pathogens.

The term *antigen* was initially used to describe molecules that stimulate the production of antibodies; in fact, the term comes from a combination of the words antibody and geneerator, and a molecule that stimulates antibody production is said to be **antigenic**. However, the role of antigens is not limited to humoral immunity and the production of antibodies; antigens also play an essential role in stimulating cellular immunity, and for this reason antigens are sometimes more accurately referred to as **immunogens**. In this text, however, we will typically refer to them as antigens.

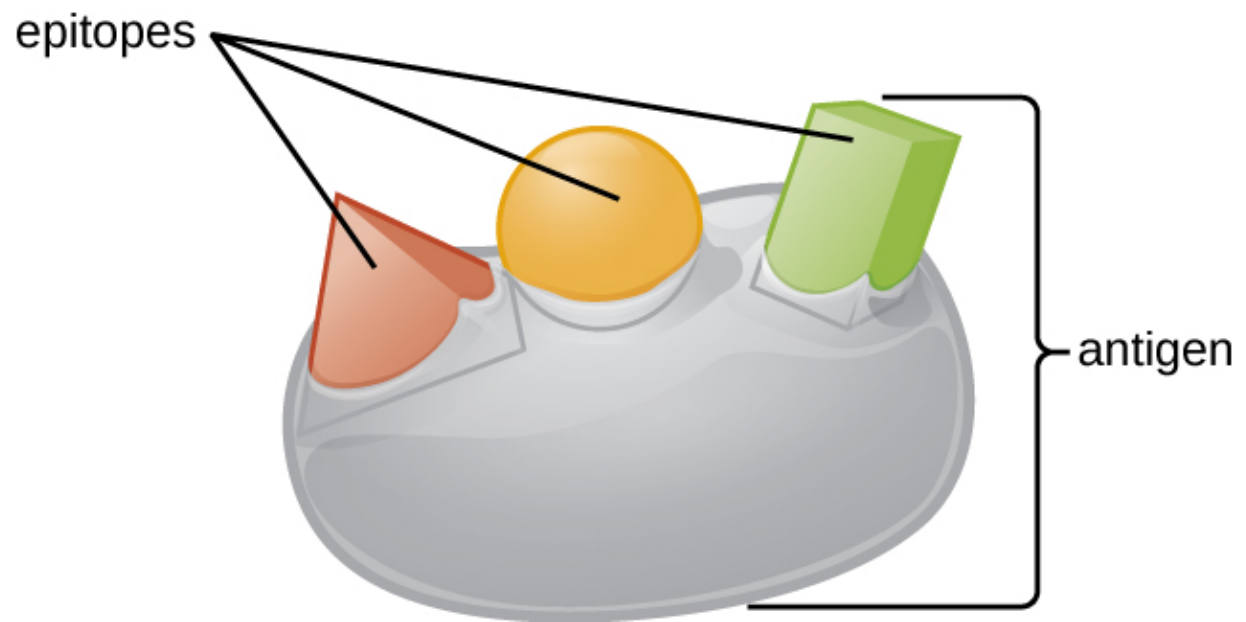
Pathogens possess a variety of structures that may contain antigens. For example, antigens from bacterial cells may be associated with their capsules, cell walls, fimbriae, flagella, or pili. Bacterial antigens may also be associated with extracellular toxins and enzymes that they secrete. Viruses possess a variety of antigens associated with their capsids, envelopes, and the spike structures they use for attachment to cells.

Antigens may belong to any number of molecular classes, including carbohydrates, lipids, nucleic acids, proteins, and combinations of these molecules. Antigens of different classes vary in their ability to stimulate adaptive immune defenses as well as in the type of response they stimulate (humoral or cellular). The structural complexity of an antigenic molecule is an important factor in its antigenic potential. In general, more complex molecules are more effective as antigens. For example, the three-dimensional complex structure of proteins make them the most effective and potent antigens, capable of stimulating both humoral and cellular immunity. In comparison, carbohydrates are less complex in structure and therefore less effective as antigens; they can only stimulate humoral immune defenses. Lipids and nucleic acids are the least antigenic

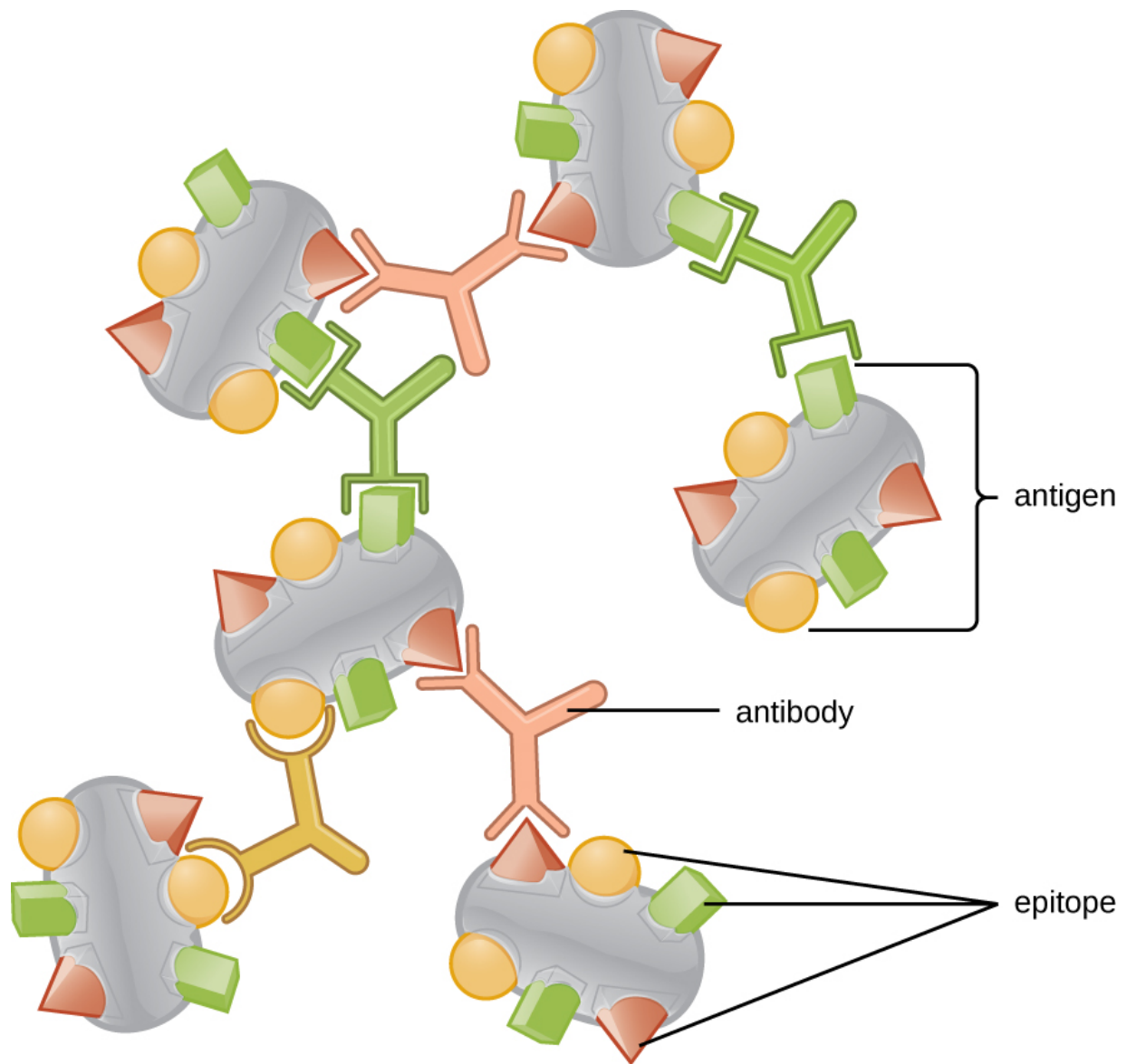
molecules, and in some cases may only become antigenic when combined with proteins or carbohydrates to form glycolipids, lipoproteins, or nucleoproteins.

One reason the three-dimensional complexity of antigens is so important is that antibodies and T cells do not recognize and interact with an entire antigen but with smaller exposed regions on the surface of antigens called **epitopes**. A single antigen may possess several different epitopes ([\[link\]](#)), and different antibodies may bind to different epitopes on the same antigen ([\[link\]](#)). For example, the bacterial flagellum is a large, complex protein structure that can possess hundreds or even thousands of epitopes with unique three-dimensional structures. Moreover, flagella from different bacterial species (or even strains of the same species) contain unique epitopes that can only be bound by specific antibodies.

An antigen's size is another important factor in its antigenic potential. Whereas large antigenic structures like flagella possess multiple epitopes, some molecules are too small to be antigenic by themselves. Such molecules, called **haptens**, are essentially free epitopes that are not part of the complex three-dimensional structure of a larger antigen. For a hapten to become antigenic, it must first attach to a larger carrier molecule (usually a protein) to produce a conjugate antigen. The hapten-specific antibodies produced in response to the conjugate antigen are then able to interact with unconjugated free hapten molecules. Haptens are not known to be associated with any specific pathogens, but they are responsible for some allergic responses. For example, the hapten urushiol, a molecule found in the oil of plants that cause poison ivy, causes an immune response that can result in a severe rash (called contact dermatitis). Similarly, the hapten penicillin can cause allergic reactions to drugs in the penicillin class.



An antigen is a macromolecule that reacts with components of the immune system. A given antigen may contain several motifs that are recognized by immune cells.



A typical protein antigen has multiple epitopes, shown by the ability of three different antibodies to bind to different epitopes of the same antigen.

Note:

- What is the difference between an antigen and an epitope?

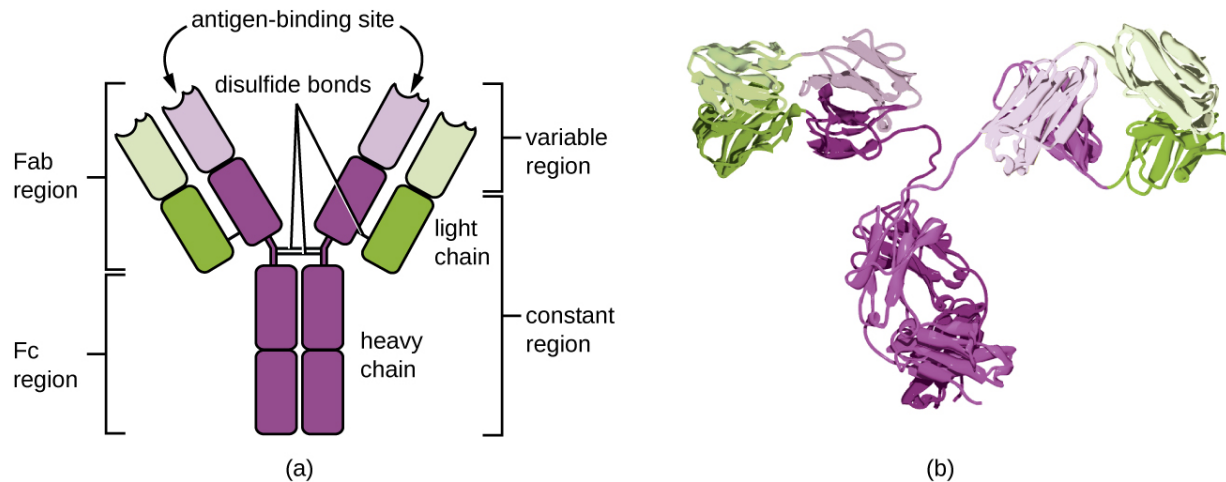
- What factors affect an antigen's antigenic potential?
- Why are haptens typically not antigenic, and how do they become antigenic?

Antibodies

Antibodies (also called immunoglobulins) are glycoproteins that are present in both the blood and tissue fluids. The basic structure of an antibody monomer consists of four protein chains held together by disulfide bonds ([link](#)). A disulfide bond is a covalent bond between the sulfhydryl *R* groups found on two cysteine amino acids. The two largest chains are identical to each other and are called the **heavy chains**. The two smaller chains are also identical to each other and are called the **light chains**. Joined together, the heavy and light chains form a basic Y-shaped structure.

The two 'arms' of the Y-shaped antibody molecule are known as the **Fab region**, for "fragment of antigen binding." The far end of the Fab region is the variable region, which serves as the site of antigen binding. The amino acid sequence in the variable region dictates the three-dimensional structure, and thus the specific three-dimensional epitope to which the Fab region is capable of binding. Although the epitope specificity of the Fab regions is identical for each arm of a single antibody molecule, this region displays a high degree of variability between antibodies with different epitope specificities. Binding to the Fab region is necessary for neutralization of pathogens, agglutination or aggregation of pathogens, and antibody-dependent cell-mediated cytotoxicity.

The constant region of the antibody molecule includes the trunk of the Y and lower portion of each arm of the Y. The trunk of the Y is also called the **Fc region**, for "fragment of crystallization," and is the site of complement factor binding and binding to phagocytic cells during antibody-mediated opsonization.



(a) The typical four-chain structure of a generic antibody monomer. (b) The corresponding three-dimensional structure of the antibody IgG. (credit b: modification of work by Tim Vickers)

Note:

- Describe the different functions of the Fab region and the Fc region.

Antibody Classes

The constant region of an antibody molecule determines its class, or isotype. The five classes of antibodies are IgG, IgM, IgA, IgD, and IgE. Each class possesses unique heavy chains designated by Greek letters γ , μ , α , δ , and ϵ , respectively. Antibody classes also exhibit important differences in abundance in serum, arrangement, body sites of action, functional roles, and size ([link](#)).

IgG is a monomer that is by far the most abundant antibody in human blood, accounting for about 80% of total serum antibody. IgG penetrates efficiently into tissue spaces, and is the only antibody class with the ability

to cross the placental barrier, providing passive immunity to the developing fetus during pregnancy. IgG is also the most versatile antibody class in terms of its role in the body's defense against pathogens.

IgM is initially produced in a monomeric membrane-bound form that serves as an antigen-binding receptor on B cells. The secreted form of IgM assembles into a pentamer with five monomers of IgM bound together by a protein structure called the J chain. Although the location of the J chain relative to the Fc regions of the five monomers prevents IgM from performing some of the functions of IgG, the ten available Fab sites associated with a pentameric IgM make it an important antibody in the body's arsenal of defenses. IgM is the first antibody produced and secreted by B cells during the primary and secondary immune responses, making pathogen-specific IgM a valuable diagnostic marker during active or recent infections.

IgA accounts for about 13% of total serum antibody, and secretory IgA is the most common and abundant antibody class found in the mucus secretions that protect the mucous membranes. IgA can also be found in other secretions such as breast milk, tears, and saliva. Secretory IgA is assembled into a dimeric form with two monomers joined by a protein structure called the secretory component. One of the important functions of secretory IgA is to trap pathogens in mucus so that they can later be eliminated from the body.

Similar to IgM, **IgD** is a membrane-bound monomer found on the surface of B cells, where it serves as an antigen-binding receptor. However, IgD is not secreted by B cells, and only trace amounts are detected in serum. These trace amounts most likely come from the degradation of old B cells and the release of IgD molecules from their cytoplasmic membranes.

IgE is the least abundant antibody class in serum. Like IgG, it is secreted as a monomer, but its role in adaptive immunity is restricted to anti-parasitic defenses. The Fc region of IgE binds to basophils and mast cells. The Fab region of the bound IgE then interacts with specific antigen epitopes, causing the cells to release potent pro-inflammatory mediators. The inflammatory reaction resulting from the activation of mast cells and

basophils aids in the defense against parasites, but this reaction is also central to allergic reactions (see [Diseases of the Immune System](#)).

The Five Immunoglobulin (Ig) Classes					
Properties	IgG monomer	IgM pentamer	Secretory IgA dimer	IgD monomer	IgE monomer
Structure					
Heavy chains	γ	μ	α	δ	ϵ
Number of antigen-binding sites	2	10	4	2	2
Molecular weight (Daltons)	150,000	900,000	385,000	180,000	200,000
Percentage of total antibody in serum	80%	6%	13% (monomer)	<1%	<1%
Crosses placenta	yes	no	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to	phagocytes				mast cells and basophils
Function	Neutralization, agglutination, complement activation, opsonization, and antibody-dependent cell-mediated cytotoxicity.	Neutralization, agglutination, and complement activation. The monomer form serves as the B-cell receptor.	Neutralization and trapping of pathogens in mucus.	B-cell receptor.	Activation of basophils and mast cells against parasites and allergens.

Note:

- What part of an antibody molecule determines its class?
- What class of antibody is involved in protection against parasites?
- Describe the difference in structure between IgM and IgG.

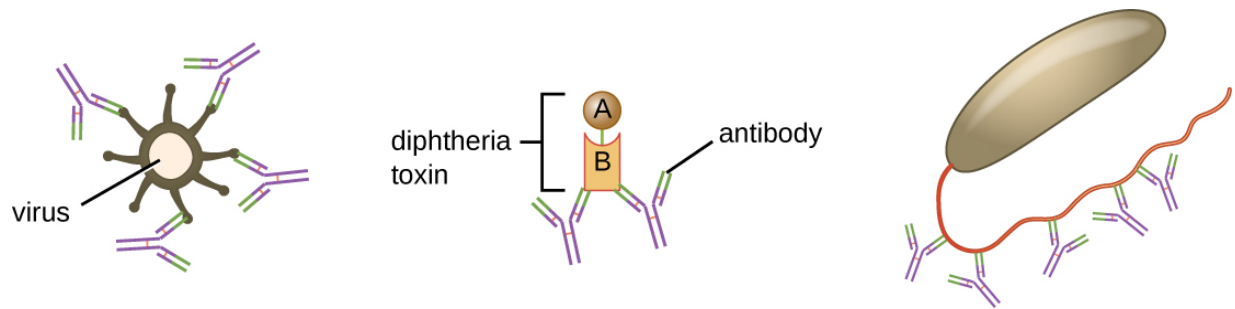
Antigen-Antibody Interactions

Different classes of antibody play important roles in the body's defense against pathogens. These functions include neutralization of pathogens, opsonization for phagocytosis, agglutination, complement activation, and antibody-dependent cell-mediated cytotoxicity. For most of these functions, antibodies also provide an important link between adaptive specific immunity and innate nonspecific immunity.

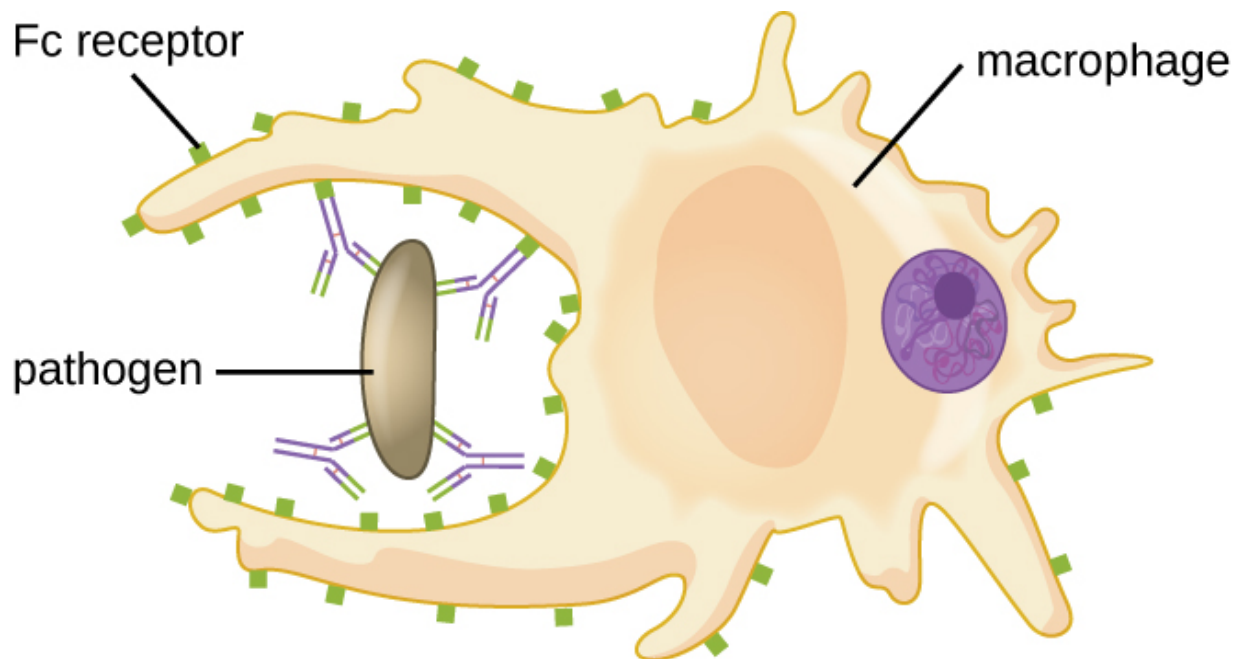
Neutralization involves the binding of certain antibodies (IgG, IgM, or IgA) to epitopes on the surface of pathogens or toxins, preventing their attachment to cells. For example, Secretory IgA can bind to specific pathogens and block initial attachment to intestinal mucosal cells. Similarly, specific antibodies can bind to certain toxins, blocking them from attaching to target cells and thus neutralizing their toxic effects. Viruses can be neutralized and prevented from infecting a cell by the same mechanism ([link](#)).

As described in [Chemical Defenses](#), opsonization is the coating of a pathogen with molecules, such as complement factors, C-reactive protein, and serum amyloid A, to assist in phagocyte binding to facilitate phagocytosis. IgG antibodies also serve as excellent opsonins, binding their Fab sites to specific epitopes on the surface of pathogens. Phagocytic cells such as macrophages, dendritic cells, and neutrophils have receptors on their surfaces that recognize and bind to the Fc portion of the IgG molecules; thus, IgG helps such phagocytes attach to and engulf the pathogens they have bound ([link](#)).

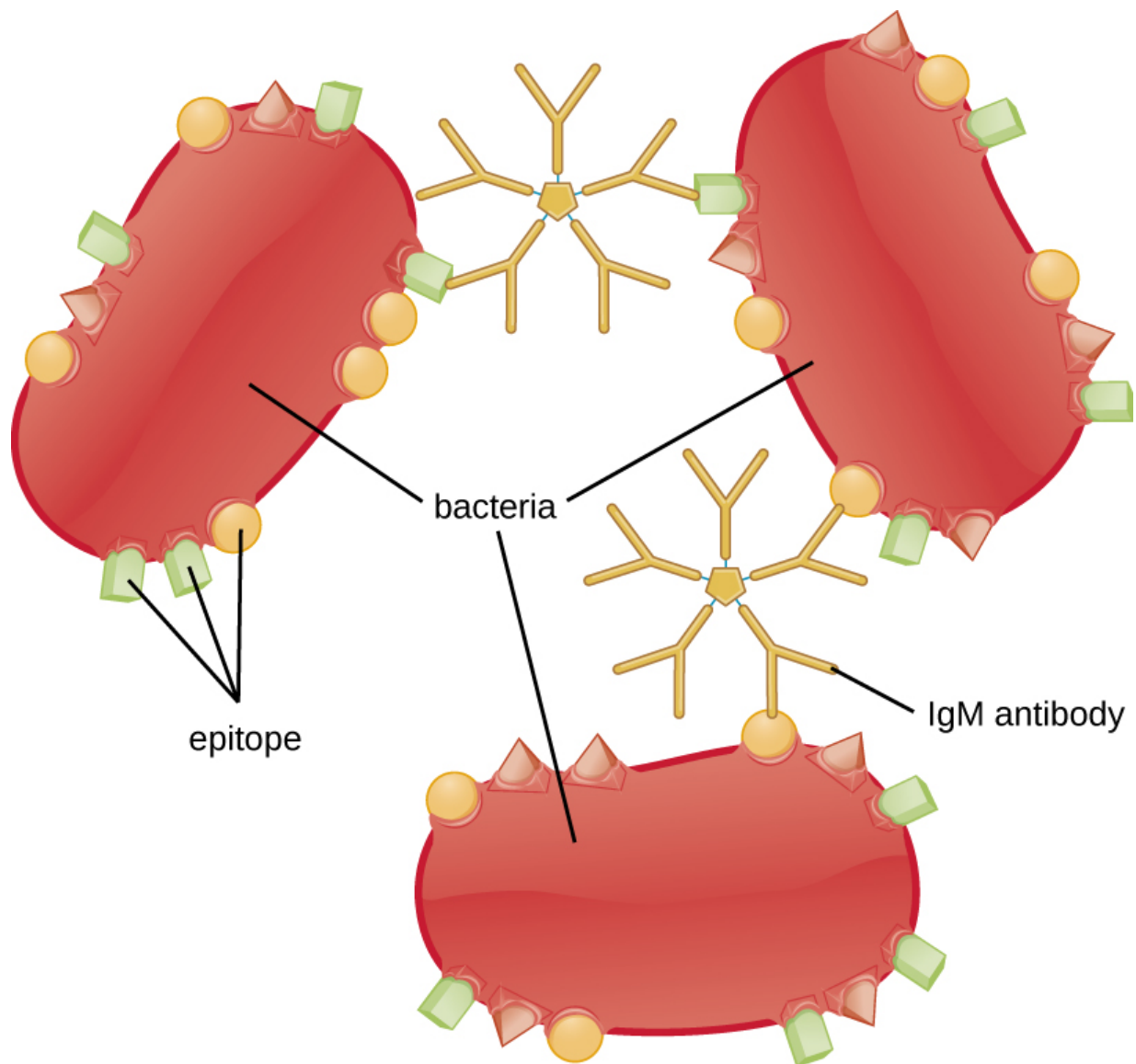
Agglutination or aggregation involves the cross-linking of pathogens by antibodies to create large aggregates ([link](#)). IgG has two Fab antigen-binding sites, which can bind to two separate pathogen cells, clumping them together. When multiple IgG antibodies are involved, large aggregates can develop; these aggregates are easier for the kidneys and spleen to filter from the blood and easier for phagocytes to ingest for destruction. The pentameric structure of IgM provides ten Fab binding sites per molecule, making it the most efficient antibody for agglutination.



Neutralization involves the binding of specific antibodies to antigens found on bacteria, viruses, and toxins, preventing them from attaching to target cells.



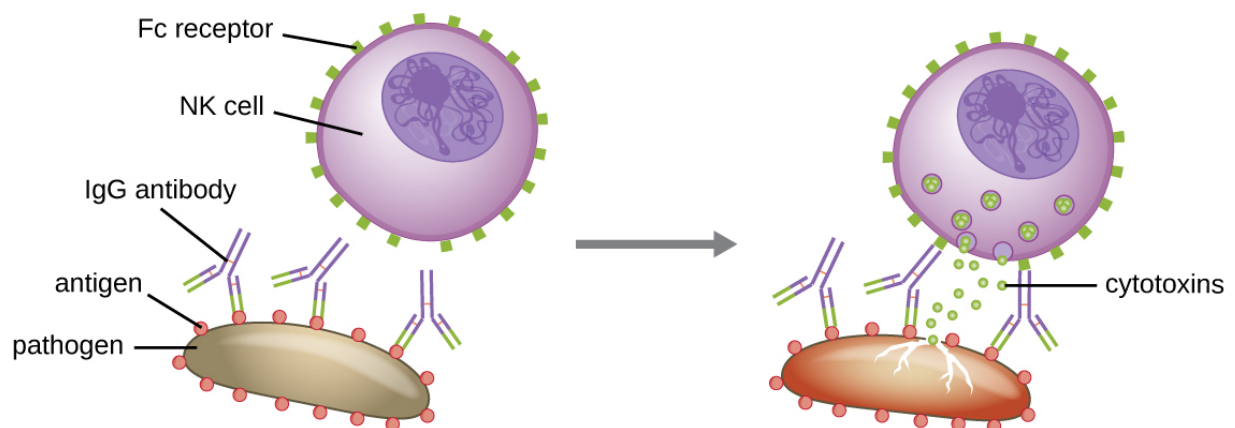
Antibodies serve as opsonins and inhibit infection by tagging pathogens for destruction by macrophages, dendritic cells, and neutrophils. These phagocytic cells use Fc receptors to bind to IgG-opsonized pathogens and initiate the first step of attachment before phagocytosis.



Antibodies, especially IgM antibodies, agglutinate bacteria by binding to epitopes on two or more bacteria simultaneously. When multiple pathogens and antibodies are present, aggregates form when the binding sites of antibodies bind with separate pathogens.

Another important function of antibodies is activation of the complement cascade. As discussed in the previous chapter, the complement system is an important component of the innate defenses, promoting the inflammatory response, recruiting phagocytes to site of infection, enhancing phagocytosis by opsonization, and killing gram-negative bacterial pathogens with the membrane attack complex (MAC). Complement activation can occur through three different pathways (see [\[link\]](#)), but the most efficient is the classical pathway, which requires the initial binding of IgG or IgM antibodies to the surface of a pathogen cell, allowing for recruitment and activation of the C1 complex.

Yet another important function of antibodies is **antibody-dependent cell-mediated cytotoxicity (ADCC)**, which enhances killing of pathogens that are too large to be phagocytosed. This process is best characterized for natural killer cells (NK cells), as shown in [\[link\]](#), but it can also involve macrophages and eosinophils. ADCC occurs when the Fab region of an IgG antibody binds to a large pathogen; Fc receptors on effector cells (e.g., NK cells) then bind to the Fc region of the antibody, bringing them into close proximity with the target pathogen. The effector cell then secretes powerful cytotoxins (e.g., perforin and granzymes) that kill the pathogen.



In this example of ADCC, antibodies bind to a large pathogenic cell that is too big for phagocytosis and then bind to Fc receptors on the membrane of a natural killer cell. This interaction brings the NK cell

into close proximity, where it can kill the pathogen through release of lethal extracellular cytotoxins.

Note:

- Where is IgA normally found?
- Which class of antibody crosses the placenta, providing protection to the fetus?
- Compare the mechanisms of opsonization and antibody-dependent cell-mediated cytotoxicity.

Key Concepts and Summary

- **Adaptive immunity** is an acquired defense against foreign pathogens that is characterized by **specificity** and **memory**. The first exposure to an antigen stimulates a **primary response**, and subsequent exposures stimulate a faster and strong **secondary response**.
- Adaptive immunity is a dual system involving **humoral immunity** (antibodies produced by B cells) and **cellular immunity** (T cells directed against intracellular pathogens).
- **Antigens**, also called **immunogens**, are molecules that activate adaptive immunity. A single antigen possesses smaller **epitopes**, each capable of inducing a specific adaptive immune response.
- An antigen's ability to stimulate an immune response depends on several factors, including its molecular class, molecular complexity, and size.
- **Antibodies (immunoglobulins)** are Y-shaped glycoproteins with two Fab sites for binding antigens and an Fc portion involved in complement activation and opsonization.
- The five classes of antibody are **IgM**, **IgG**, **IgA**, **IgE**, and **IgD**, each differing in size, arrangement, location within the body, and function. The five primary functions of antibodies are neutralization,

opsonization, agglutination, complement activation, and antibody-dependent cell-mediated cytotoxicity (ADCC).

Multiple Choice

Exercise:

Problem: Antibodies are produced by _____.

- a. plasma cells
- b. T cells
- c. bone marrow
- d. Macrophages

Solution:

A

Exercise:

Problem: Cellular adaptive immunity is carried out by _____.

- a. B cells
- b. T cells
- c. bone marrow
- d. neutrophils

Solution:

B

Exercise:

Problem:

A single antigen molecule may be composed of many individual _____.

- a. T-cell receptors
- b. B-cell receptors
- c. MHC II
- d. epitopes

Solution:

D

Exercise:

Problem: Which class of molecules is the most antigenic?

- a. polysaccharides
- b. lipids
- c. proteins
- d. carbohydrates

Solution:

C

Matching

Exercise:

Problem: Match the antibody class with its description.

___IgA	A. This class of antibody is the only one that can cross the placenta.
--------	--

___IgD	B. This class of antibody is the first to appear after activation of B cells.
___IgE	C. This class of antibody is involved in the defense against parasitic infections and involved in allergic responses.
___IgG	D. This class of antibody is found in very large amounts in mucus secretions.
___IgM	E. This class of antibody is not secreted by B cells but is expressed on the surface of naïve B cells.

Solution:

d, e, c, a, b

Fill in the Blank

Exercise:

Problem:

There are two critically important aspects of adaptive immunity. The first is specificity, while the second is _____.

Solution:

memory

Exercise:

Problem:

_____ immunity involves the production of antibody molecules that bind to specific antigens.

Solution:

Humoral

Exercise:**Problem:**

The heavy chains of an antibody molecule contain _____ region segments, which help to determine its class or isotype.

Solution:

constant

Exercise:**Problem:**

The variable regions of the heavy and light chains form the _____ sites of an antibody.

Solution:

antigen-binding

Short Answer**Exercise:****Problem:**

What is the difference between humoral and cellular adaptive immunity?

Exercise:

Problem: What is the difference between an antigen and a hapten?

Exercise:

Problem:

Describe the mechanism of antibody-dependent cell-mediated cytotoxicity.

Major Histocompatibility Complexes and Antigen-Presenting Cells

LEARNING OBJECTIVES

- Identify cells that express MHC I and/or MHC II molecules and describe the structures and cellular location of MHC I and MHC II molecules
- Identify the cells that are antigen-presenting cells
- Describe the process of antigen processing and presentation with MHC I and MHC II

As discussed in [Cellular Defenses](#), major histocompatibility complex (MHC) molecules are expressed on the surface of healthy cells, identifying them as normal and “self” to natural killer (NK) cells. MHC molecules also play an important role in the presentation of foreign antigens, which is a critical step in the activation of T cells and thus an important mechanism of the adaptive immune system.

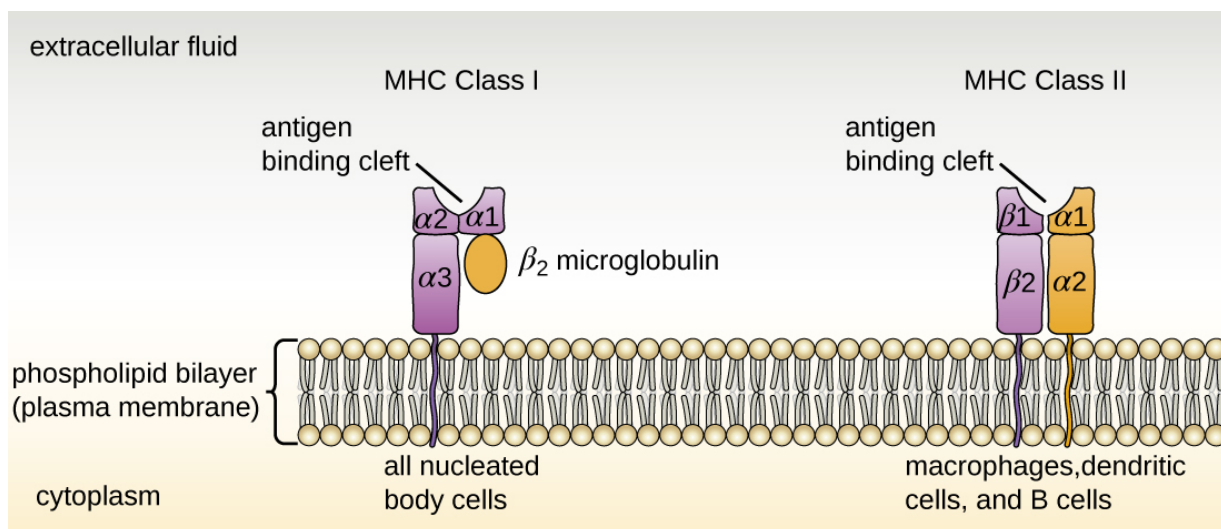
Major Histocompatibility Complex Molecules

The **major histocompatibility complex (MHC)** is a collection of genes coding for MHC molecules found on the surface of all nucleated cells of the body. In humans, the MHC genes are also referred to as human leukocyte antigen (HLA) genes. Mature red blood cells, which lack a nucleus, are the only cells that do not express MHC molecules on their surface.

There are two classes of MHC molecules involved in adaptive immunity, MHC I and MHC II ([\[link\]](#)). **MHC I** molecules are found on all nucleated cells; they present normal self-antigens as well as abnormal or nonself

pathogens to the effector T cells involved in cellular immunity. In contrast, **MHC II** molecules are only found on macrophages, dendritic cells, and B cells; they present abnormal or nonself pathogen antigens for the initial activation of T cells.

Both types of MHC molecules are transmembrane glycoproteins that assemble as dimers in the cytoplasmic membrane of cells, but their structures are quite different. MHC I molecules are composed of a longer α protein chain coupled with a smaller β_2 microglobulin protein, and only the α chain spans the cytoplasmic membrane. The α chain of the MHC I molecule folds into three separate domains: α_1 , α_2 and α_3 . MHC II molecules are composed of two protein chains (an α and a β chain) that are approximately similar in length. Both chains of the MHC II molecule possess portions that span the plasma membrane, and each chain folds into two separate domains: α_1 and α_2 , and β_1 , and β_2 . In order to present abnormal or non-self-antigens to T cells, MHC molecules have a cleft that serves as the antigen-binding site near the “top” (or outermost) portion of the MHC-I or MHC-II dimer. For MHC I, the antigen-binding cleft is formed by the α_1 and α_2 domains, whereas for MHC II, the cleft is formed by the α_1 and β_1 domains ([\[link\]](#)).



MHC I are found on all nucleated body cells, and MHC II are found on macrophages, dendritic cells, and B cells (along with MHC I). The

antigen-binding cleft of MHC I is formed by domains α_1 and α_2 . The antigen-binding cleft of MHC II is formed by domains α_1 and β_1 .

Note:

- Compare the structures of the MHC I and MHC II molecules.

Antigen-Presenting Cells (APCs)

All nucleated cells in the body have mechanisms for processing and presenting antigens in association with MHC molecules. This signals the immune system, indicating whether the cell is normal and healthy or infected with an intracellular pathogen. However, only macrophages, dendritic cells, and B cells have the ability to present antigens specifically for the purpose of activating T cells; for this reason, these types of cells are sometimes referred to as **antigen-presenting cells (APCs)**.

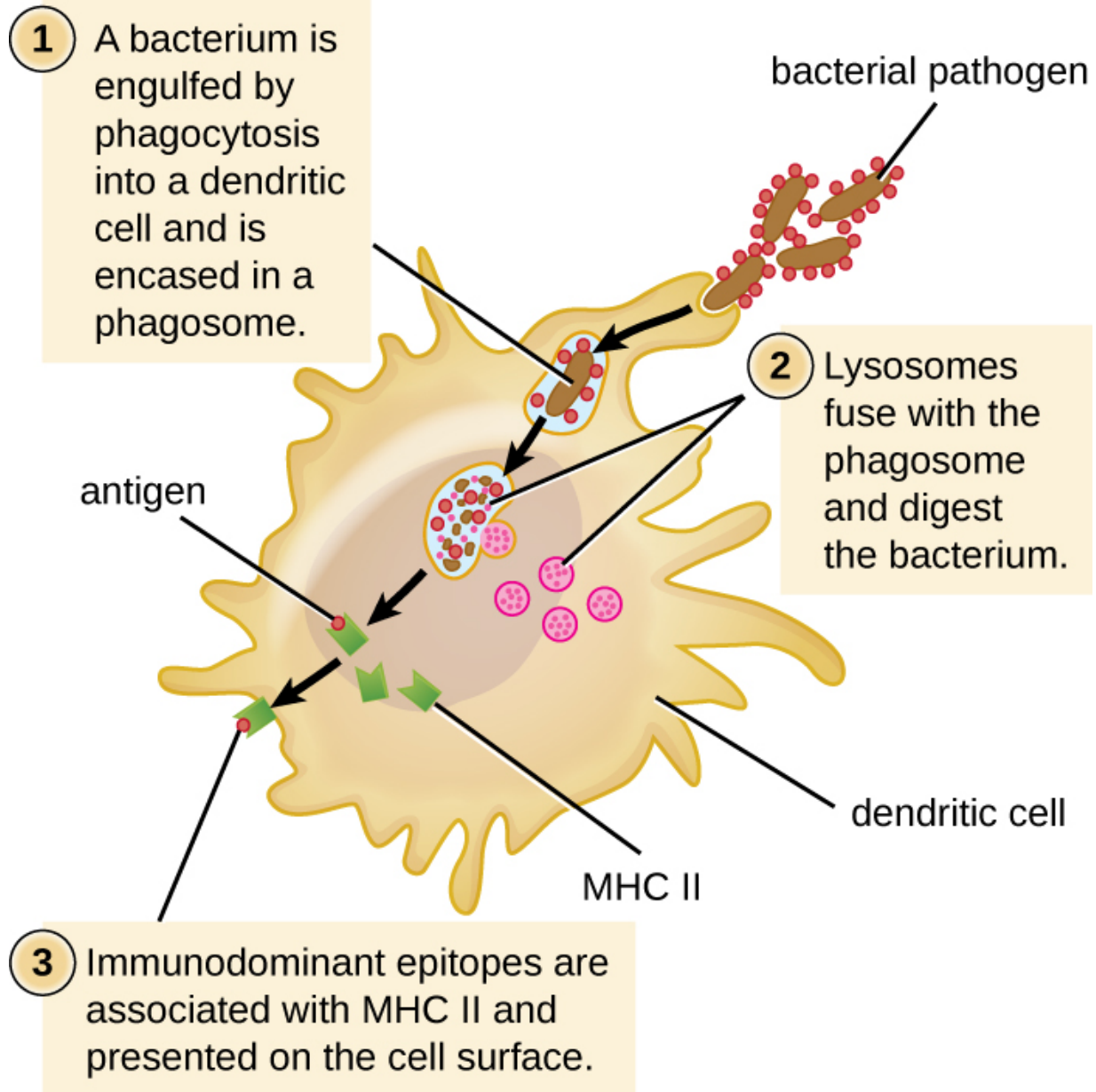
While all APCs play a similar role in adaptive immunity, there are some important differences to consider. Macrophages and dendritic cells are phagocytes that ingest and kill pathogens that penetrate the first-line barriers (i.e., skin and mucous membranes). B cells, on the other hand, do not function as phagocytes but play a primary role in the production and secretion of antibodies. In addition, whereas macrophages and dendritic cells recognize pathogens through nonspecific receptor interactions (e.g., PAMPs, toll-like receptors, and receptors for opsonizing complement or antibody), B cells interact with foreign pathogens or their free antigens using antigen-specific immunoglobulin as receptors (monomeric IgD and IgM). When the immunoglobulin receptors bind to an antigen, the B cell internalizes the antigen by endocytosis before processing and presenting the antigen to T cells.

Antigen Presentation with MHC II Molecules

MHC II molecules are only found on the surface of APCs. Macrophages and dendritic cells use similar mechanisms for processing and presentation of antigens and their epitopes in association with MHC II; B cells use somewhat different mechanisms that will be described further in [B Lymphocytes and Humoral Immunity](#). For now, we will focus on the steps of the process as they pertain to dendritic cells.

After a dendritic cell recognizes and attaches to a pathogen cell, the pathogen is internalized by phagocytosis and is initially contained within a phagosome. Lysosomes containing antimicrobial enzymes and chemicals fuse with the phagosome to create a phagolysosome, where degradation of the pathogen for antigen processing begins. Proteases (protein-degrading) are especially important in antigen processing because only protein antigen epitopes are presented to T cells by MHC II ([link](#)).

APCs do not present all possible epitopes to T cells; only a selection of the most antigenic or immunodominant epitopes are presented. The mechanism by which epitopes are selected for processing and presentation by an APC is complicated and not well understood; however, once the most antigenic, immunodominant epitopes have been processed, they associate within the antigen-binding cleft of MHC II molecules and are translocated to the cell surface of the dendritic cell for presentation to T cells.



A dendritic cell phagocytoses a bacterial cell and brings it into a phagosome. Lysosomes fuse with the phagosome to create a phagolysosome, where antimicrobial chemicals and enzymes degrade the bacterial cell. Proteases process bacterial antigens, and the most antigenic epitopes are selected and presented on the cell's surface in conjunction with MHC II molecules. T cells recognize the presented antigens and are thus activated.

Note:

- What are the three kinds of APCs?
- What role to MHC II molecules play in antigen presentation?
- What is the role of antigen presentation in adaptive immunity?

Antigen Presentation with MHC I Molecules

MHC I molecules, found on all normal, healthy, nucleated cells, signal to the immune system that the cell is a normal “self” cell. In a healthy cell, proteins normally found in the cytoplasm are degraded by proteasomes (enzyme complexes responsible for degradation and processing of proteins) and processed into self-antigen epitopes; these self-antigen epitopes bind within the MHC I antigen-binding cleft and are then presented on the cell surface. Immune cells, such as NK cells, recognize these self-antigens and do not target the cell for destruction. However, if a cell becomes infected with an intracellular pathogen (e.g., a virus), protein antigens specific to the pathogen are processed in the proteasomes and bind with MHC I molecules for presentation on the cell surface. This presentation of pathogen-specific antigens with MHC I signals that the infected cell must be targeted for destruction along with the pathogen.

Before elimination of infected cells can begin, APCs must first activate the T cells involved in cellular immunity. If an intracellular pathogen directly infects the cytoplasm of an APC, then the processing and presentation of antigens can occur as described (in proteasomes and on the cell surface with MHC I). However, if the intracellular pathogen does not directly infect APCs, an alternative strategy called **cross-presentation** is utilized. In cross-presentation, antigens are brought into the APC by mechanisms normally leading to presentation with MHC II (i.e., through phagocytosis), but the antigen is presented on an MHC I molecule for CD8 T cells. The exact mechanisms by which cross-presentation occur are not yet well understood, but it appears that cross-presentation is primarily a function of dendritic cells and not macrophages or B cells.

Note:

- Compare and contrast antigen processing and presentation associated with MHC I and MHC II molecules.
- What is cross-presentation, and when is it likely to occur?

Key Concepts and Summary

- **Major histocompatibility complex (MHC)** is a collection of genes coding for glycoprotein molecules expressed on the surface of all nucleated cells.
- **MHC I** molecules are expressed on all nucleated cells and are essential for presentation of normal “self” antigens. Cells that become infected by intracellular pathogens can present foreign antigens on MHC I as well, marking the infected cell for destruction.
- **MHC II** molecules are expressed only on the surface of **antigen-presenting cells** (macrophages, dendritic cells, and B cells). Antigen presentation with MHC II is essential for the activation of T cells.
- **Antigen-presenting cells (APCs)** primarily ingest pathogens by phagocytosis, destroy them in the phagolysosomes, process the protein antigens, and select the most antigenic/immunodominant epitopes with MHC II for presentation to T cells.
- **Cross-presentation** is a mechanism of antigen presentation and T-cell activation used by dendritic cells not directly infected by the pathogen; it involves phagocytosis of the pathogen but presentation on MHC I rather than MHC II.

Multiple Choice**Exercise:**

Problem: MHC I molecules present

- a. processed foreign antigens from proteasomes.

- b. processed self-antigens from phagolysosome.
- c. antibodies.
- d. T cell antigens.

Solution:

A

Exercise:

Problem: MHC II molecules present

- a. processed self-antigens from proteasomes.
- b. processed foreign antigens from phagolysosomes.
- c. antibodies.
- d. T cell receptors.

Solution:

B

Exercise:

Problem:

Which type of antigen-presenting molecule is found on all nucleated cells?

- a. MHC II
- b. MHC I
- c. antibodies
- d. B-cell receptors

Solution:

B

Exercise:**Problem:**

Which type of antigen-presenting molecule is found only on macrophages, dendritic cells, and B cells?

- a. MHC I
- b. MHC II
- c. T-cell receptors
- d. B-cell receptors

Solution:

B

Fill in the Blank**Exercise:**

Problem: MHC molecules are used for antigen _____ to T cells.

Solution:

presentation

Exercise:**Problem:**

MHC II molecules are made up of two subunits (α and β) of approximately equal size, whereas MHC I molecules consist of a larger α subunit and a smaller subunit called _____.

Solution:

β_2 microglobulin

Critical Thinking

Exercise:

Problem:

Which mechanism of antigen presentation would be used to present antigens from a cell infected with a virus?

Exercise:

Problem:

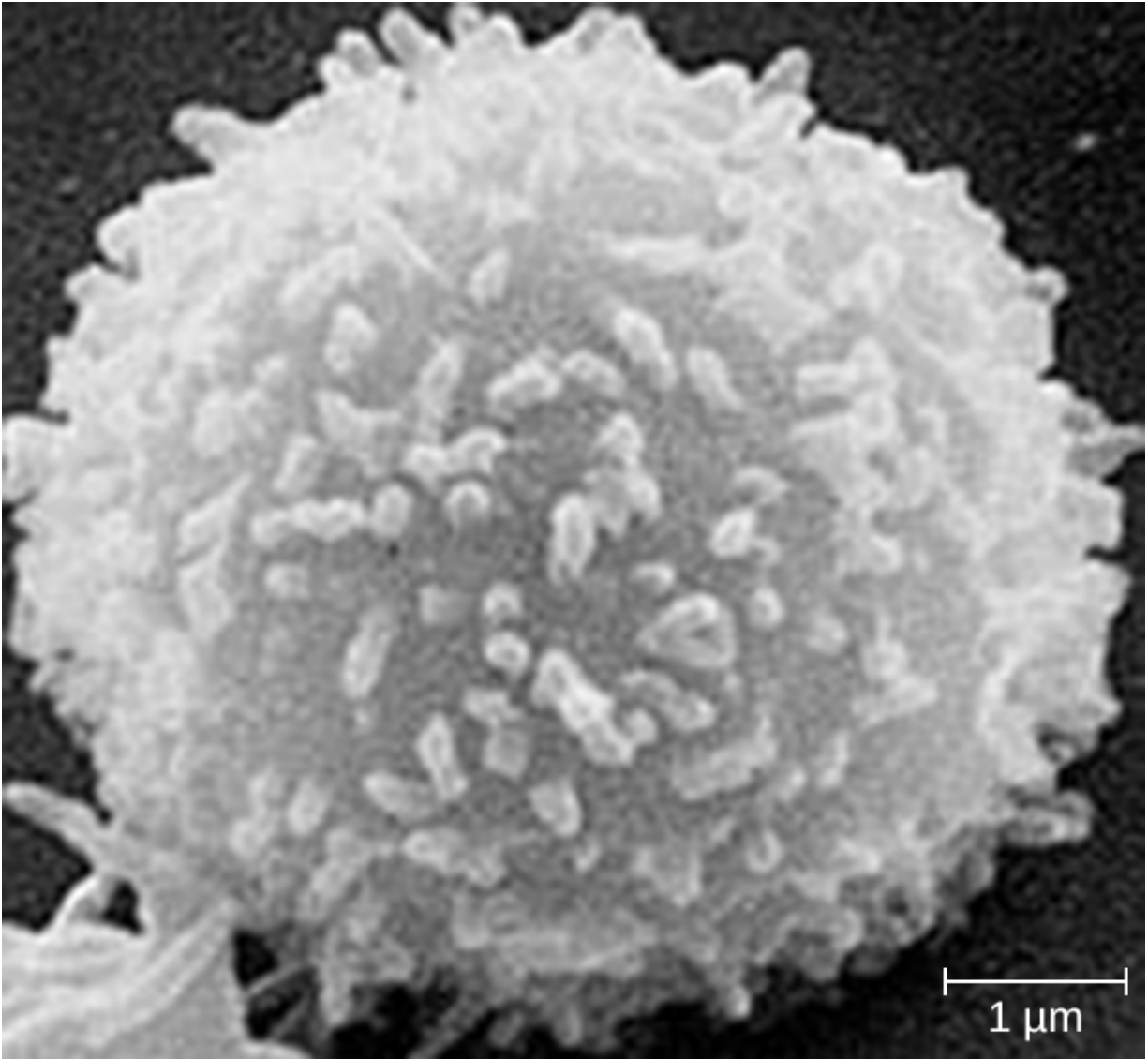
Which pathway of antigen presentation would be used to present antigens from an extracellular bacterial infection?

T Lymphocytes and Cellular Immunity

LEARNING OBJECTIVES

- Describe the process of T-cell maturation and thymic selection
- Explain the genetic events that lead to diversity of T-cell receptors
- Compare and contrast the various classes and subtypes of T cells in terms of activation and function
- Explain the mechanism by which superantigens effect unregulated T-cell activation

As explained in [Overview of Specific Adaptive Immunity](#), the antibodies involved in humoral immunity often bind pathogens and toxins before they can attach to and invade host cells. Thus, humoral immunity is primarily concerned with fighting pathogens in extracellular spaces. However, pathogens that have already gained entry to host cells are largely protected from the humoral antibody-mediated defenses. Cellular immunity, on the other hand, targets and eliminates intracellular pathogens through the actions of T lymphocytes, or T cells ([\[link\]](#)). T cells also play a more central role in orchestrating the overall adaptive immune response (humoral as well as cellular) along with the cellular defenses of innate immunity.



This scanning electron micrograph shows a T lymphocyte, which is responsible for the cell-mediated immune response. The spike-like membrane structures increase surface area, allowing for greater interaction with other cell types and their signals. (credit: modification of work by NCI)

T Cell Production and Maturation

T cells, like all other white blood cells involved in innate and adaptive immunity, are formed from multipotent hematopoietic stem cells (HSCs) in the bone marrow (see [\[link\]](#)). However, unlike the white blood cells of innate immunity, eventual T cells differentiate first into lymphoid stem cells that then become small, immature lymphocytes, sometimes called lymphoblasts. The first steps of differentiation occur in the red marrow of bones ([\[link\]](#)), after which immature T lymphocytes enter the bloodstream and travel to the thymus for the final steps of maturation ([\[link\]](#)). Once in the thymus, the immature T lymphocytes are referred to as thymocytes.

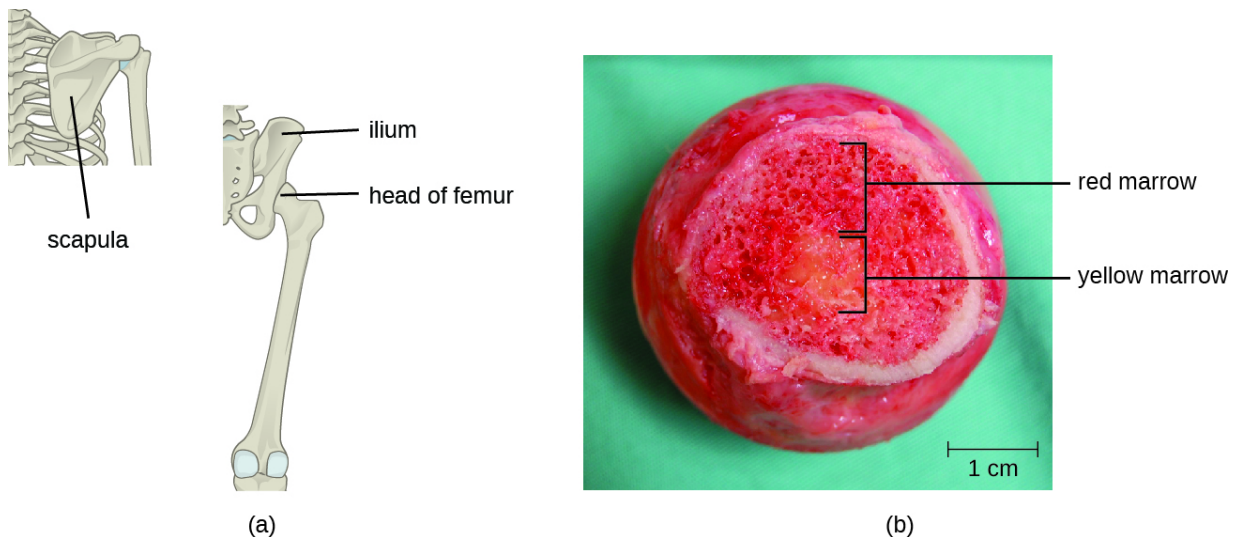
The maturation of thymocytes within the thymus can be divided into three critical steps of positive and negative selection, collectively referred to as **thymic selection**. The first step of thymic selection occurs in the cortex of the thymus and involves the development of a functional T-cell receptor (TCR) that is required for activation by APCs. Thymocytes with defective TCRs are removed by negative selection through the induction of **apoptosis** (programmed controlled cell death). The second step of thymic selection also occurs in the cortex and involves the positive selection of thymocytes that will interact appropriately with MHC molecules. Thymocytes that can interact appropriately with MHC molecules receive a positive stimulation that moves them further through the process of maturation, whereas thymocytes that do not interact appropriately are not stimulated and are eliminated by apoptosis. The third and final step of thymic selection occurs in both the cortex and medulla and involves negative selection to remove self-reacting thymocytes, those that react to self-antigens, by apoptosis. This final step is sometimes referred to as **central tolerance** because it prevents self-reacting T cells from reaching the bloodstream and potentially causing autoimmune disease, which occurs when the immune system attacks healthy “self” cells.

Despite central tolerance, some self-reactive T cells generally escape the thymus and enter the peripheral bloodstream. Therefore, a second line of defense called **peripheral tolerance** is needed to protect against autoimmune disease. Peripheral tolerance involves mechanisms of **anergy** and inhibition of self-reactive T cells by **regulatory T cells**. Anergy refers to a state of nonresponsiveness to antigen stimulation. In the case of self-reactive T cells that escape the thymus, lack of an essential co-stimulatory

signal required for activation causes anergy and prevents autoimmune activation. Regulatory T cells participate in peripheral tolerance by inhibiting the activation and function of self-reactive T cells and by secreting anti-inflammatory cytokines.

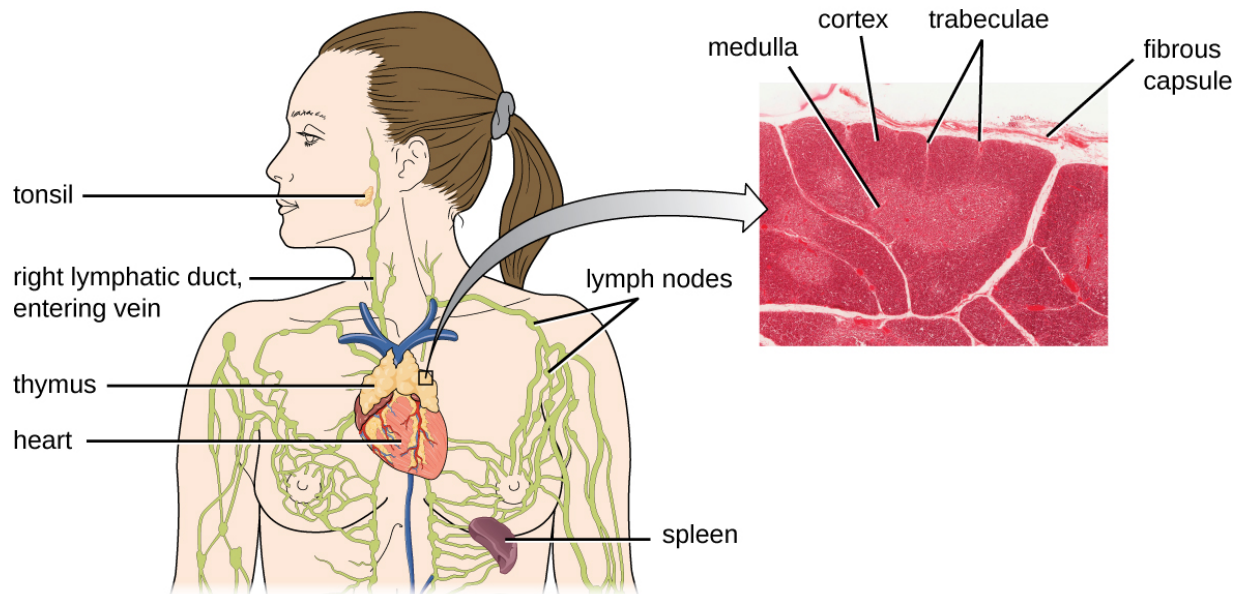
It is not completely understood what events specifically direct maturation of thymocytes into regulatory T cells. Current theories suggest the critical events may occur during the third step of thymic selection, when most self-reactive T cells are eliminated. Regulatory T cells may receive a unique signal that is below the threshold required to target them for negative selection and apoptosis. Consequently, these cells continue to mature and then exit the thymus, armed to inhibit the activation of self-reactive T cells.

It has been estimated that the three steps of thymic selection eliminate 98% of thymocytes. The remaining 2% that exit the thymus migrate through the bloodstream and lymphatic system to sites of secondary lymphoid organs/tissues, such as the lymph nodes, spleen, and tonsils ([\[link\]](#)), where they await activation through the presentation of specific antigens by APCs. Until they are activated, they are known as **mature naïve T cells**.



(a) Red bone marrow can be found in the head of the femur (thighbone) and is also present in the flat bones of the body, such as the ilium and the scapula. (b) Red bone marrow is the site of

production and differentiation of many formed elements of blood, including erythrocytes, leukocytes, and platelets. The yellow bone marrow is populated primarily with adipose cells.



The thymus is a bi-lobed, H-shaped glandular organ that is located just above the heart. It is surrounded by a fibrous capsule of connective tissue. The darkly staining cortex and the lighter staining medulla of individual lobules are clearly visible in the light micrograph of the thymus of a newborn (top right, LM $\times$ 100). (credit micrograph: modification of micrograph provided by the Regents of University of Michigan Medical School © 2012)

Note:

- What anatomical sites are involved in T cell production and maturation?
- What are the three steps involved in thymic selection?
- Why are central tolerance and peripheral tolerance important? What do they prevent?

Classes of T Cells

T cells can be categorized into three distinct classes: helper T cells, regulatory T cells, and cytotoxic T cells. These classes are differentiated based on their expression of certain surface molecules, their mode of activation, and their functional roles in adaptive immunity ([link](#)).

All T cells produce **cluster of differentiation (CD) molecules**, cell surface glycoproteins that can be used to identify and distinguish between the various types of white blood cells. Although T cells can produce a variety of CD molecules, CD4 and CD8 are the two most important used for differentiation of the classes. Helper T cells and regulatory T cells are characterized by the expression of CD4 on their surface, whereas cytotoxic T cells are characterized by the expression of CD8.

Classes of T cells can also be distinguished by the specific MHC molecules and APCs with which they interact for activation. Helper T cells and regulatory T cells can only be activated by APCs presenting antigens associated with MHC II. In contrast, cytotoxic T cells recognize antigens presented in association with MHC I, either by APCs or by nucleated cells infected with an intracellular pathogen.

The different classes of T cells also play different functional roles in the immune system. **Helper T cells** serve as the central orchestrators that help activate and direct functions of humoral and cellular immunity. In addition, helper T cells enhance the pathogen-killing functions of macrophages and NK cells of innate immunity. In contrast, the primary role of regulatory T cells is to prevent undesirable and potentially damaging immune responses. Their role in peripheral tolerance, for example, protects against autoimmune

disorders, as discussed earlier. Finally, **cytotoxic T cells** are the primary effector cells for cellular immunity. They recognize and target cells that have been infected by intracellular pathogens, destroying infected cells along with the pathogens inside.

Classes of T Cells			
Class	Surface CD Molecules	Activation	Functions
Helper T cells	CD4	APCs presenting antigens associated with MHC II	Orchestrate humoral and cellular immunity
			Involved in the activation of macrophages and NK cells
Regulatory T cells	CD4	APCs presenting antigens associated with MHC II	Involved in peripheral tolerance and prevention of autoimmune responses

Classes of T Cells			
Class	Surface CD Molecules	Activation	Functions
Cytotoxic T cells	CD8	APCs or infected nucleated cells presenting antigens associated with MHC I	Destroy cells infected with intracellular pathogens

Note:

- What are the unique functions of the three classes of T cells?
- Which T cells can be activated by antigens presented by cells other than APCs?

T-Cell Receptors

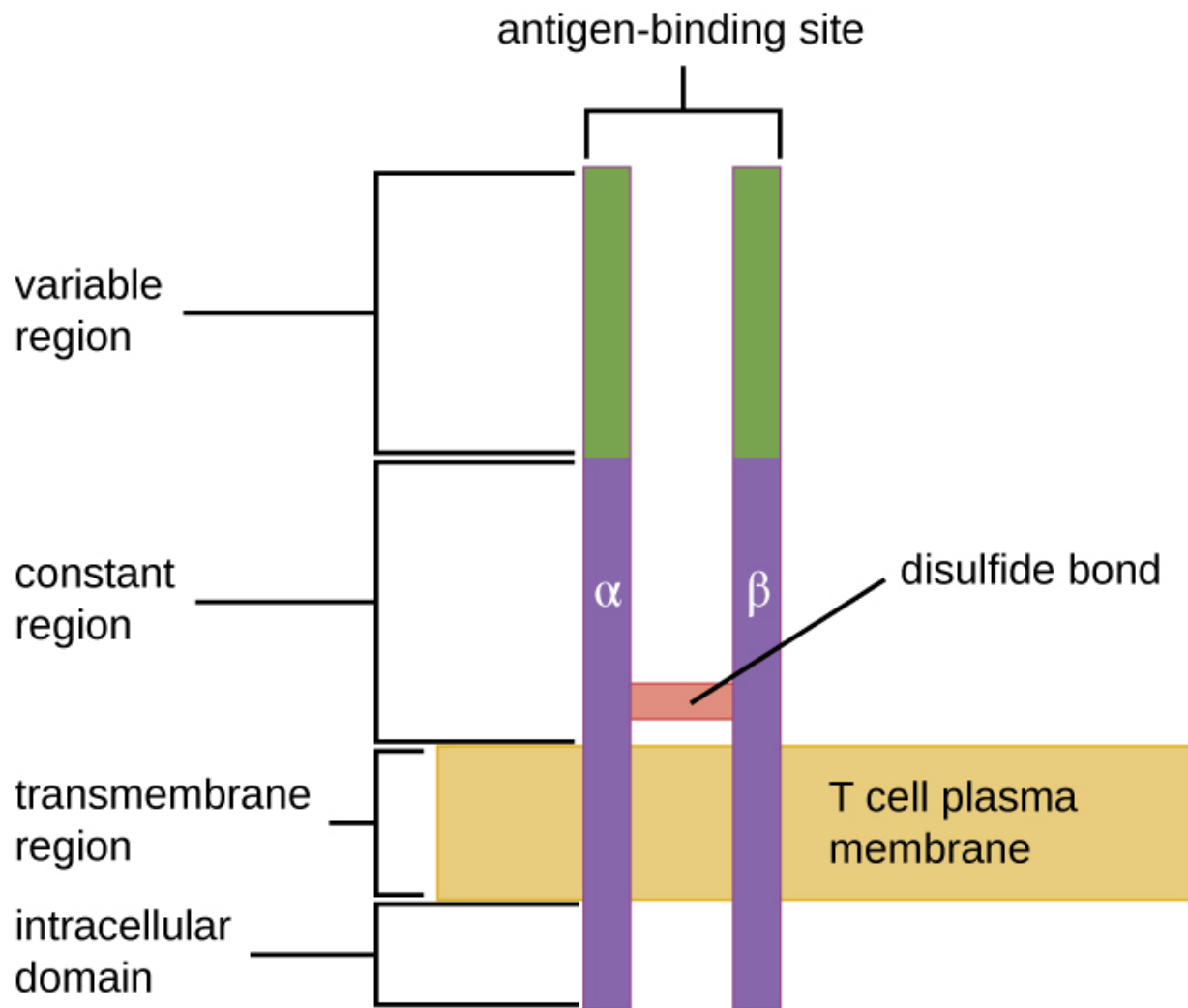
For both helper T cells and cytotoxic T cells, activation is a complex process that requires the interactions of multiple molecules and exposure to cytokines. The **T-cell receptor (TCR)** is involved in the first step of pathogen epitope recognition during the activation process.

The TCR comes from the same receptor family as the antibodies IgD and IgM, the antigen receptors on the B cell membrane surface, and thus shares common structural elements. Similar to antibodies, the TCR has a variable region and a constant region, and the variable region provides the antigen-binding site ([\[link\]](#)). However, the structure of TCR is smaller and less

complex than the immunoglobulin molecules ([\[link\]](#)). Whereas immunoglobulins have four peptide chains and Y-shaped structures, the TCR consists of just two peptide chains (α and β chains), both of which span the cytoplasmic membrane of the T cell.

TCRs are epitope-specific, and it has been estimated that 25 million T cells with unique epitope-binding TCRs are required to protect an individual against a wide range of microbial pathogens. Because the human genome only contains about 25,000 genes, we know that each specific TCR cannot be encoded by its own set of genes. This raises the question of how such a vast population of T cells with millions of specific TCRs can be achieved. The answer is a process called genetic rearrangement, which occurs in the thymus during the first step of thymic selection.

The genes that code for the variable regions of the TCR are divided into distinct gene segments called variable (V), diversity (D), and joining (J) segments. The genes segments associated with the α chain of the TCR consist 70 or more different V_α segments and 61 different J_α segments. The gene segments associated with the β chain of the TCR consist of 52 different V_β segments, two different D_β segments, and 13 different J_β segments. During the development of the functional TCR in the thymus, genetic rearrangement in a T cell brings together one V_α segment and one J_α segment to code for the variable region of the α chain. Similarly, genetic rearrangement brings one of the V_β segments together with one of the D_β segments and one of the J_β segments to code for the variable region of the β chain. All the possible combinations of rearrangements between different segments of V, D, and J provide the genetic diversity required to produce millions of TCRs with unique epitope-specific variable regions.



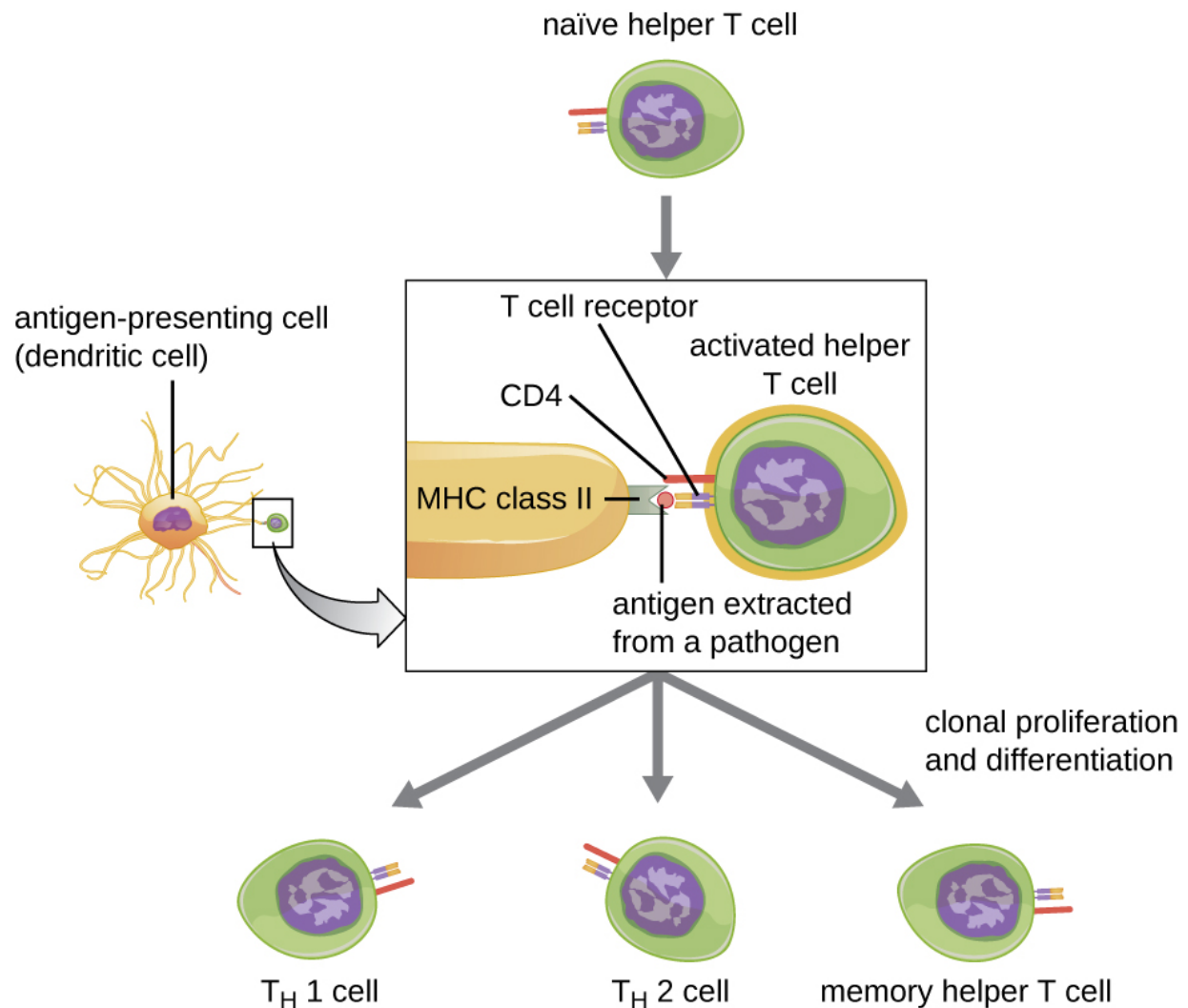
A T-cell receptor spans the cytoplasmic membrane and projects variable binding regions into the extracellular space to bind processed antigens associated with MHC I or MHC II molecules.

Note:

- What are the similarities and differences between TCRs and immunoglobulins?
- What process is used to provide millions of unique TCR binding sites?

Activation and Differentiation of Helper T Cells

Helper T cells can only be activated by APCs presenting processed foreign epitopes in association with MHC II. The first step in the activation process is TCR recognition of the specific foreign epitope presented within the MHC II antigen-binding cleft. The second step involves the interaction of CD4 on the helper T cell with a region of the MHC II molecule separate from the antigen-binding cleft. This second interaction anchors the MHC II-TCR complex and ensures that the helper T cell is recognizing both the foreign (“nonself”) epitope and “self” antigen of the APC; both recognitions are required for activation of the cell. In the third step, the APC and T cell secrete cytokines that activate the helper T cell. The activated helper T cell then proliferates, dividing by mitosis to produce clonal naïve helper T cells that differentiate into subtypes with different functions ([\[link\]](#)).



This illustration depicts the activation of a naïve (unactivated) helper T cell by an antigen-presenting cell and the subsequent proliferation and differentiation of the activated T cell into different subtypes.

Activated helper T cells can differentiate into one of four distinct subtypes, summarized in [\[link\]](#). The differentiation process is directed by APC-secreted cytokines. Depending on which APC-secreted cytokines interact with an activated helper T cell, the cell may differentiate into a T helper 1 (T_H1) cell, a T helper 2 (T_H2) cell, or a memory helper T cell. The two types of helper T cells are relatively short-lived **effector cells**, meaning that they perform various functions of the immediate immune response. In

contrast, **memory helper T cells** are relatively long lived; they are programmed to “remember” a specific antigen or epitope in order to mount a rapid, strong, secondary response to subsequent exposures.

T_H1 cells secrete their own cytokines that are involved in stimulating and orchestrating other cells involved in adaptive and innate immunity. For example, they stimulate cytotoxic T cells, enhancing their killing of infected cells and promoting differentiation into memory cytotoxic T cells. T_H1 cells also stimulate macrophages and neutrophils to become more effective in their killing of intracellular bacteria. They can also stimulate NK cells to become more effective at killing target cells.

T_H2 cells play an important role in orchestrating the humoral immune response through their secretion of cytokines that activate B cells and direct B cell differentiation and antibody production. Various cytokines produced by T_H2 cells orchestrate antibody class switching, which allows B cells to switch between the production of IgM, IgG, IgA, and IgE as needed to carry out specific antibody functions and to provide pathogen-specific humoral immune responses.

A third subtype of helper T cells called **T_H17 cells** was discovered through observations that immunity to some infections is not associated with T_H1 or T_H2 cells. T_H17 cells and the cytokines they produce appear to be specifically responsible for the body’s defense against chronic mucocutaneous infections. Patients who lack sufficient T_H17 cells in the mucosa (e.g., HIV patients) may be more susceptible to bacteremia and gastrointestinal infections. [\[footnote\]](#)

Blaschitz C., Raffatellu M. “Th17 cytokines and the gut mucosal barrier.” J Clin Immunol. 2010 Mar; 30(2):196-203. doi: 10.1007/s10875-010-9368-7.

Subtypes of Helper T Cells

Subtypes of Helper T cells	Functions
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Subtype	Functions
T _H 1 cells	Stimulate cytotoxic T cells and produce memory cytotoxic T cells
	Stimulate macrophages and neutrophils (PMNs) for more effective intracellular killing of pathogens
	Stimulate NK cells to kill more effectively
T _H 2 cells	Stimulate B cell activation and differentiation into plasma cells and memory B cells
	Direct antibody class switching in B cells
T _H 17 cells	Stimulate immunity to specific infections such as chronic mucocutaneous infections
Memory helper T cells	“Remember” a specific pathogen and mount a strong, rapid secondary response upon re-exposure

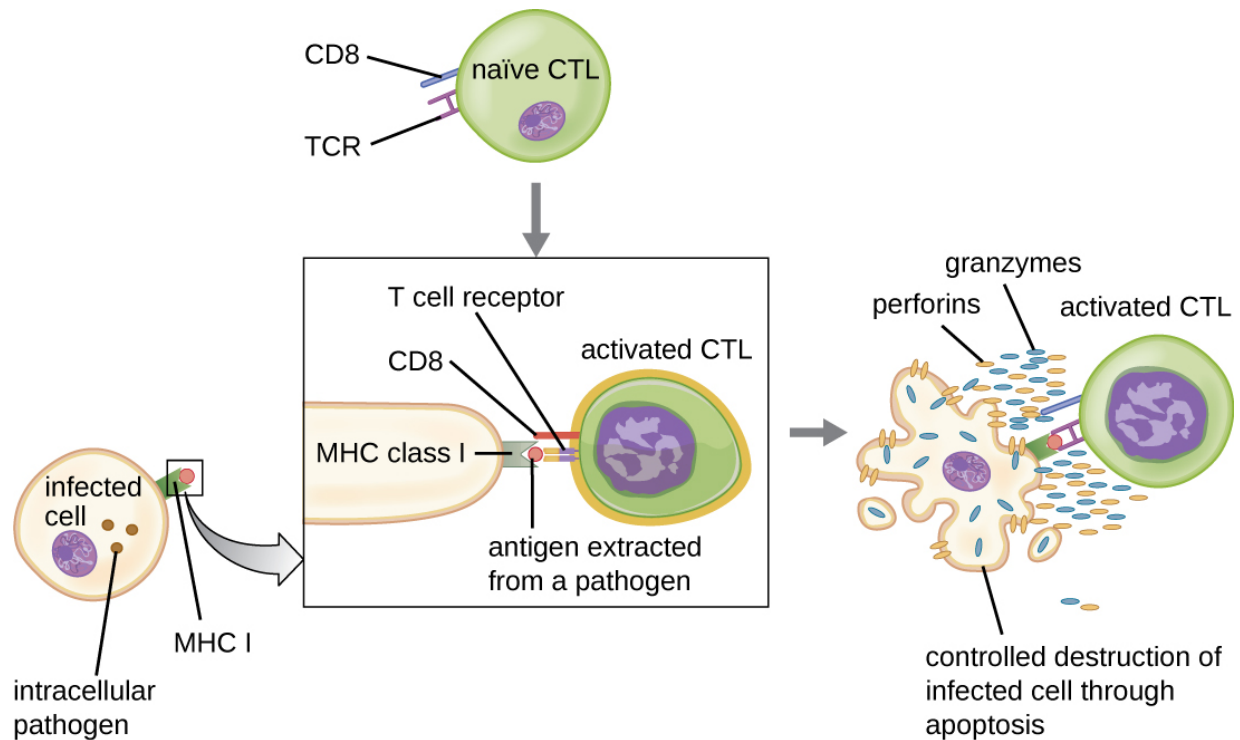
Activation and Differentiation of Cytotoxic T Cells

Cytotoxic T cells (also referred to as cytotoxic T lymphocytes, or CTLs) are activated by APCs in a three-step process similar to that of helper T cells. The key difference is that the activation of cytotoxic T cells involves recognition of an antigen presented with MHC I (as opposed to MHC II) and interaction of CD8 (as opposed to CD4) with the receptor complex. After the successful co-recognition of foreign epitope and self-antigen, the production of cytokines by the APC and the cytotoxic T cell activate clonal proliferation and differentiation. Activated cytotoxic T cells can

differentiate into effector cytotoxic T cells that target pathogens for destruction or memory cells that are ready to respond to subsequent exposures.

As noted, proliferation and differentiation of cytotoxic T cells is also stimulated by cytokines secreted from T_H1 cells activated by the same foreign epitope. The co-stimulation that comes from these T_H1 cells is provided by secreted cytokines. Although it is possible for activation of cytotoxic T cells to occur without stimulation from T_H1 cells, the activation is not as effective or long-lasting.

Once activated, cytotoxic T cells serve as the effector cells of cellular immunity, recognizing and kill cells infected with intracellular pathogens through a mechanism very similar to that of NK cells. However, whereas NK cells recognize nonspecific signals of cell stress or abnormality, cytotoxic T cells recognize infected cells through antigen presentation of pathogen-specific epitopes associated with MHC I. Once an infected cell is recognized, the TCR of the cytotoxic T cell binds to the epitope and releases perforin and granzymes that destroy the infected cell ([\[link\]](#)). Perforin is a protein that creates pores in the target cell, and **granzymes** are proteases that enter the pores and induce apoptosis. This mechanism of programmed cell death is a controlled and efficient means of destroying and removing infected cells without releasing the pathogens inside to infect neighboring cells, as might occur if the infected cells were simply lysed.



This figure illustrates the activation of a naïve (unactivated) cytotoxic T cell (CTL) by an antigen-presenting MHC I molecule on an infected body cell. Once activated, the CTL releases perforin and granzymes that invade the infected cell and induce controlled cell death, or apoptosis.

Note:

In this [video](#), you can see a cytotoxic T cell inducing apoptosis in a target cell.

Note:

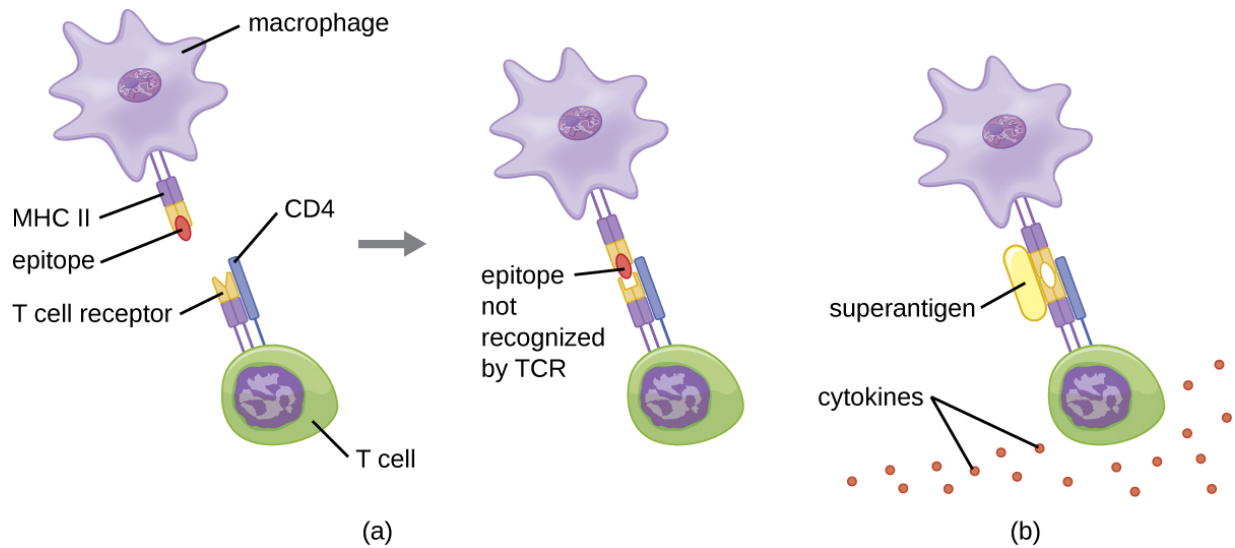
- Compare and contrast the activation of helper T cells and cytotoxic T cells.

- What are the different functions of helper T cell subtypes?
- What is the mechanism of CTL-mediated destruction of infected cells?

Superantigens and Unregulated Activation of T Cells

When T cell activation is controlled and regulated, the result is a protective response that is effective in combating infections. However, if T cell activation is unregulated and excessive, the result can be a life-threatening. Certain bacterial and viral pathogens produce toxins known as superantigens (see [Virulence Factors of Bacterial and Viral Pathogens](#)) that can trigger such an unregulated response. Known bacterial superantigens include toxic shock syndrome toxin (TSST), staphylococcal enterotoxins, streptococcal pyrogenic toxins, streptococcal superantigen, and the streptococcal mitogenic exotoxin. Viruses known to produce superantigens include Epstein-Barr virus (human herpesvirus 4), cytomegalovirus (human herpesvirus 5), and others.

The mechanism of T cell activation by superantigens involves their simultaneous binding to MHC II molecules of APCs and the variable region of the TCR β chain. This binding occurs outside of the antigen-binding cleft of MHC II, so the superantigen will bridge together and activate MHC II and TCR without specific foreign epitope recognition ([\[link\]](#)). The result is an excessive, uncontrolled release of cytokines, often called a **cytokine storm**, which stimulates an excessive inflammatory response. This can lead to a dangerous decrease in blood pressure, shock, multi-organ failure, and potentially, death.



(a) The macrophage in this figure is presenting a foreign epitope that does not match the TCR of the T cell. Because the T cell does not recognize the epitope, it is not activated. (b) The macrophage in this figure is presenting a superantigen that is not recognized by the TCR of the T cell, yet the superantigen still is able to bridge and bind the MHC II and TCR molecules. This nonspecific, uncontrolled activation of the T cell results in an excessive release of cytokines that activate other T cells and cause excessive inflammation. (credit: modification of work by “Microbiotic”/YouTube)

Note:

- What are examples of superantigens?
- How does a superantigen activate a helper T cell?
- What effect does a superantigen have on a T cell?

Note:

Superantigens

Melissa, an otherwise healthy 22-year-old woman, is brought to the emergency room by her concerned boyfriend. She complains of a sudden onset of high fever, vomiting, diarrhea, and muscle aches. In her initial interview, she tells the attending physician that she is on hormonal birth control and also is two days into the menstruation portion of her cycle. She is on no other medications and is not abusing any drugs or alcohol. She is not a smoker. She is not diabetic and does not currently have an infection of any kind to her knowledge.

While waiting in the emergency room, Melissa's blood pressure begins to drop dramatically and her mental state deteriorates to general confusion. The physician believes she is likely suffering from toxic shock syndrome (TSS). TSS is caused by the toxin TSST-1, a superantigen associated with *Staphylococcus aureus*, and improper tampon use is a common cause of infections leading to TSS. The superantigen inappropriately stimulates widespread T cell activation and excessive cytokine release, resulting in a massive and systemic inflammatory response that can be fatal.

Vaginal or cervical swabs may be taken to confirm the presence of the microbe, but these tests are not critical to perform based on Melissa's symptoms and medical history. The physician prescribes rehydration, supportive therapy, and antibiotics to stem the bacterial infection. She also prescribes drugs to increase Melissa's blood pressure. Melissa spends three days in the hospital undergoing treatment; in addition, her kidney function is monitored because of the high risk of kidney failure associated with TSS. After 72 hours, Melissa is well enough to be discharged to continue her recovery at home.

- In what way would antibiotic therapy help to combat a superantigen?

Note:

Part 2

Olivia's swollen lymph nodes, abdomen, and spleen suggest a strong immune response to a systemic infection in progress. In addition, little Olivia is reluctant to turn her head and appears to be experiencing severe neck pain. The physician orders a complete blood count, blood culture, and

lumbar puncture. The cerebrospinal fluid (CSF) obtained appears cloudy and is further evaluated by Gram stain assessment and culturing for potential bacterial pathogens. The complete blood count indicates elevated numbers of white blood cells in Olivia's bloodstream. The white blood cell increases are recorded at 28.5 K/ μ L (normal range: 6.0–17.5 K/ μ L). The neutrophil percentage was recorded as 60% (normal range: 23–45%). Glucose levels in the CSF were registered at 30 mg/100 mL (normal range: 50–80 mg/100 mL). The WBC count in the CSF was 1,163/ mm^3 (normal range: 5–20/ mm^3).

- Based on these results, do you have a preliminary diagnosis?
- What is a recommended treatment based on this preliminary diagnosis?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Immature T lymphocytes are produced in the red bone marrow and travel to the thymus for maturation.
- **Thymic selection** is a three-step process of negative and positive selection that determines which T cells will mature and exit the thymus into the peripheral bloodstream.
- **Central tolerance** involves negative selection of self-reactive T cells in the thymus, and **peripheral tolerance** involves **anergy** and **regulatory T cells** that prevent self-reactive immune responses and autoimmunity.
- The **TCR** is similar in structure to immunoglobulins, but less complex. Millions of unique epitope-binding TCRs are encoded through a process of genetic rearrangement of V, D, and J gene segments.
- T cells can be divided into three classes—**helper T cells**, **cytotoxic T cells**, and **regulatory T cells**—based on their expression of CD4 or CD8, the MHC molecules with which they interact for activation, and their respective functions.

- Activated helper T cells differentiate into **T_H1, T_H2, T_H17**, or **memory T cell subtypes**. Differentiation is directed by the specific cytokines to which they are exposed. T_H1, T_H2, and T_H17 perform different functions related to stimulation of adaptive and innate immune defenses. Memory T cells are long-lived cells that can respond quickly to secondary exposures.
- Once activated, cytotoxic T cells target and kill cells infected with intracellular pathogens. Killing requires recognition of specific pathogen epitopes presented on the cell surface using MHC I molecules. Killing is mediated by **perforin** and **granzymes** that induce apoptosis.
- **Superantigens** are bacterial or viral proteins that cause a nonspecific activation of helper T cells, leading to an excessive release of cytokines (**cytokine storm**) and a systemic, potentially fatal inflammatory response.

Multiple Choice

Exercise:

Problem: What is a superantigen?

- a protein that is highly efficient at stimulating a single type of productive and specific T cell response
- a protein produced by antigen-presenting cells to enhance their presentation capabilities
- a protein produced by T cells as a way of increasing the antigen activation they receive from antigen-presenting cells
- a protein that activates T cells in a nonspecific and uncontrolled manner

Solution:

D

Exercise:

Problem: To what does the TCR of a helper T cell bind?

- a. antigens presented with MHC I molecules
- b. antigens presented with MHC II molecules
- c. free antigen in a soluble form
- d. haptens only

Solution:

B

Exercise:

Problem:

Cytotoxic T cells will bind with their TCR to which of the following?

- a. antigens presented with MHC I molecules
- b. antigens presented with MHC II molecules
- c. free antigen in a soluble form
- d. haptens only

Solution:

A

Exercise:

Problem:

A _____ molecule is a glycoprotein used to identify and distinguish white blood cells.

- a. T-cell receptor
- b. B-cell receptor
- c. MHC I
- d. cluster of differentiation

Solution:

D

Exercise:

Problem:

Name the T helper cell subset involved in antibody production.

- a. T_H1
- b. T_H2
- c. T_H17
- d. CTL

Solution:

B

Fill in the Blank

Exercise:

Problem:

A _____ T cell will become activated by presentation of foreign antigen associated with an MHC I molecule.

Solution:

cytotoxic

Exercise:

Problem:

A _____ T cell will become activated by presentation of foreign antigen in association with an MHC II molecule.

Solution:

helper

Exercise:**Problem:**

A TCR is a protein dimer embedded in the plasma membrane of a T cell. The _____ region of each of the two protein chains is what gives it the capability to bind to a presented antigen.

Solution:

variable

Exercise:**Problem:**

Peripheral tolerance mechanisms function on T cells after they mature and exit the _____.

Solution:

thymus

Exercise:**Problem:**

Both _____ and effector T cells are produced during differentiation of activated T cells.

Solution:

memory

Short Answer

Exercise:

Problem:

What is the basic difference in effector function between helper and cytotoxic T cells?

Exercise:

Problem:

What necessary interactions are required for activation of helper T cells and activation/effector function of cytotoxic T cells?

B Lymphocytes and Humoral Immunity

LEARNING OBJECTIVES

- Describe the production and maturation of B cells
- Compare the structure of B-cell receptors and T-cell receptors
- Compare T-dependent and T-independent activation of B cells
- Compare the primary and secondary antibody responses

Humoral immunity refers to mechanisms of the adaptive immune defenses that are mediated by antibodies secreted by B lymphocytes, or B cells. This section will focus on B cells and discuss their production and maturation, receptors, and mechanisms of activation.

B Cell Production and Maturation

Like T cells, B cells are formed from multipotent hematopoietic stem cells (HSCs) in the bone marrow and follow a pathway through lymphoid stem cell and lymphoblast (see [\[link\]](#)). Unlike T cells, however, lymphoblasts destined to become B cells do not leave the bone marrow and travel to the thymus for maturation. Rather, eventual B cells continue to mature in the bone marrow.

The first step of B cell maturation is an assessment of the functionality of their antigen-binding receptors. This occurs through positive selection for B cells with normal functional receptors. A mechanism of negative selection is then used to eliminate self-reacting B cells and minimize the risk of autoimmunity. Negative selection of self-reacting B cells can involve elimination by apoptosis, editing or modification of the receptors so they

are no longer self-reactive, or induction of anergy in the B cell. Immature B cells that pass the selection in the bone marrow then travel to the spleen for their final stages of maturation. There they become **naïve mature B cells**, i.e., mature B cells that have not yet been activated.

Note:

- Compare the maturation of B cells with the maturation of T cells.

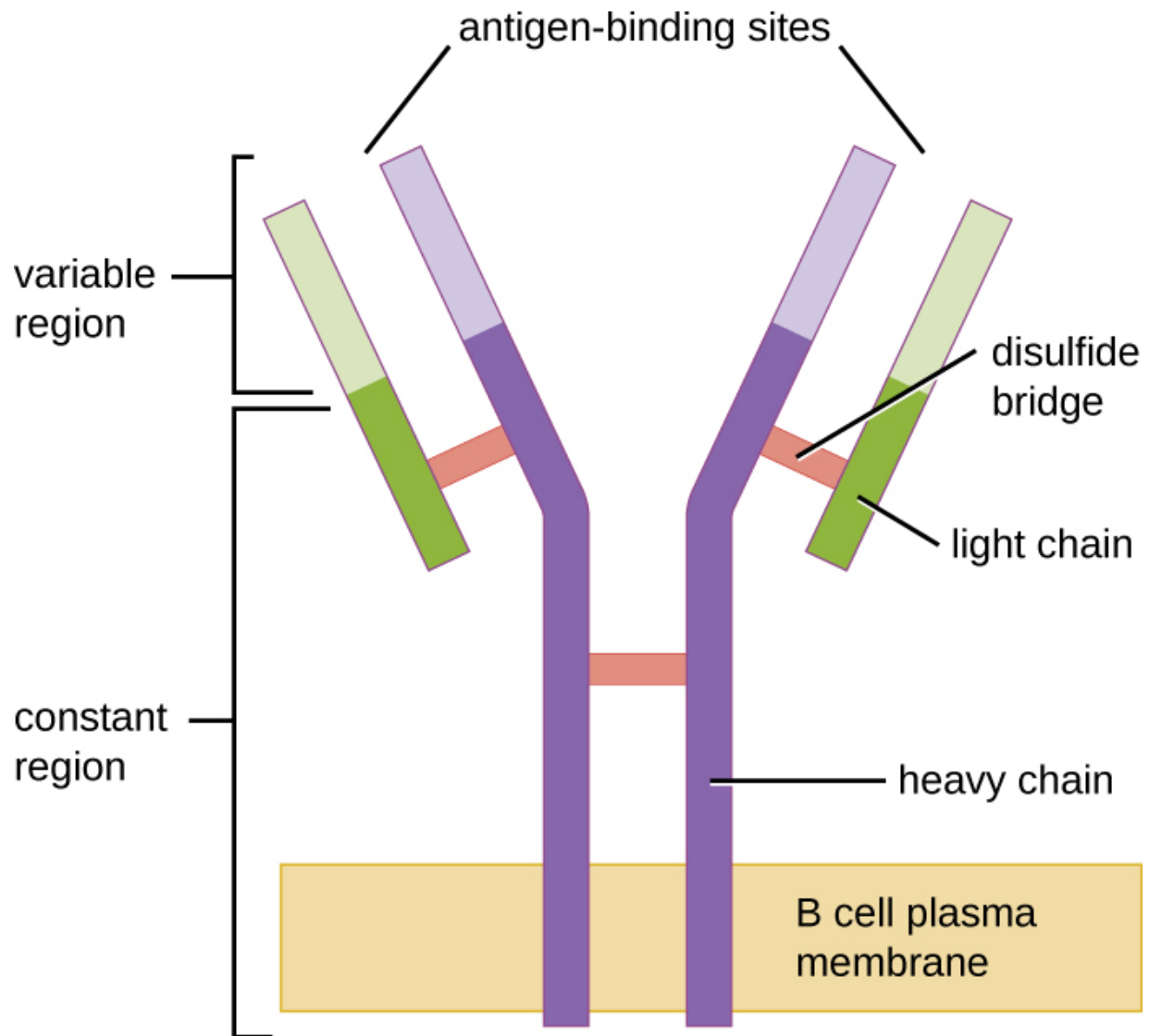
B-Cell Receptors

Like T cells, B cells possess antigen-specific receptors with diverse specificities. Although they rely on T cells for optimum function, B cells can be activated without help from T cells. **B-cell receptors (BCRs)** for naïve mature B cells are membrane-bound monomeric forms of IgD and IgM. They have two identical heavy chains and two identical light chains connected by disulfide bonds into a basic “Y” shape ([\[link\]](#)). The trunk of the Y-shaped molecule, the constant region of the two heavy chains, spans the B cell membrane. The two antigen-binding sites exposed to the exterior of the B cell are involved in the binding of specific pathogen epitopes to initiate the activation process. It is estimated that each naïve mature B cell has upwards of 100,000 BCRs on its membrane, and each of these BCRs has an identical epitope-binding specificity.

In order to be prepared to react to a wide range of microbial epitopes, B cells, like T cells, use genetic rearrangement of hundreds of gene segments to provide the necessary diversity of receptor specificities. The variable region of the BCR heavy chain is made up of V, D, and J segments, similar to the β chain of the TCR. The variable region of the BCR light chain is made up of V and J segments, similar to the α chain of the TCR. Genetic rearrangement of all possible combinations of V-J-D (heavy chain) and V-J (light chain) provides for millions of unique antigen-binding sites for the BCR and for the antibodies secreted after activation.

One important difference between BCRs and TCRs is the way they can interact with antigenic epitopes. Whereas TCRs can only interact with antigenic epitopes that are presented within the antigen-binding cleft of MHC I or MHC II, BCRs do not require antigen presentation with MHC; they can interact with epitopes on free antigens or with epitopes displayed on the surface of intact pathogens. Another important difference is that TCRs only recognize protein epitopes, whereas BCRs can recognize epitopes associated with different molecular classes (e.g., proteins, polysaccharides, lipopolysaccharides).

Activation of B cells occurs through different mechanisms depending on the molecular class of the antigen. Activation of a B cell by a protein antigen requires the B cell to function as an APC, presenting the protein epitopes with MHC II to helper T cells. Because of their dependence on T cells for activation of B cells, protein antigens are classified as **T-dependent antigens**. In contrast, polysaccharides, lipopolysaccharides, and other nonprotein antigens are considered **T-independent antigens** because they can activate B cells without antigen processing and presentation to T cells.



B-cell receptors are embedded in the membranes of B cells. The variable regions of all of the receptors on a single cell bind the same specific antigen.

Note:

- What types of molecules serve as the BCR?

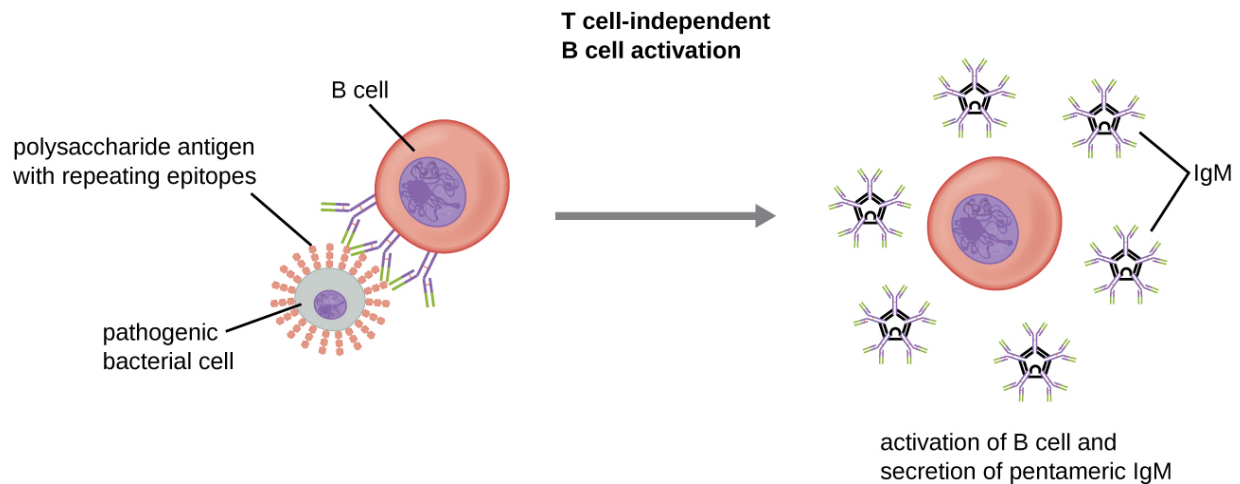
- What are the differences between TCRs and BCRs with respect to antigen recognition?
- Which molecule classes are T-dependent antigens and which are T-independent antigens?

T Cell-Independent Activation of B cells

Activation of B cells without the cooperation of helper T cells is referred to as T cell-independent activation and occurs when BCRs interact with T-independent antigens. T-independent antigens (e.g., polysaccharide capsules, lipopolysaccharide) have repetitive epitope units within their structure, and this repetition allows for the cross-linkage of multiple BCRs, providing the first signal for activation ([\[link\]](#)). Because T cells are not involved, the second signal has to come from other sources, such as interactions of toll-like receptors with PAMPs or interactions with factors from the complement system.

Once a B cell is activated, it undergoes clonal proliferation and daughter cells differentiate into plasma cells. **Plasma cells** are antibody factories that secrete large quantities of antibodies. After differentiation, the surface BCRs disappear and the plasma cell secretes pentameric IgM molecules that have the same antigen specificity as the BCRs ([\[link\]](#)).

The T cell-independent response is short-lived and does not result in the production of memory B cells. Thus it will not result in a secondary response to subsequent exposures to T-independent antigens.



T-independent antigens have repeating epitopes that can induce B cell recognition and activation without involvement from T cells. A second signal, such as interaction of TLRs with PAMPs (not shown), is also required for activation of the B cell. Once activated, the B cell proliferates and differentiates into antibody-secreting plasma cells.

Note:

- What are the two signals required for T cell-independent activation of B cells?
- What is the function of a plasma cell?

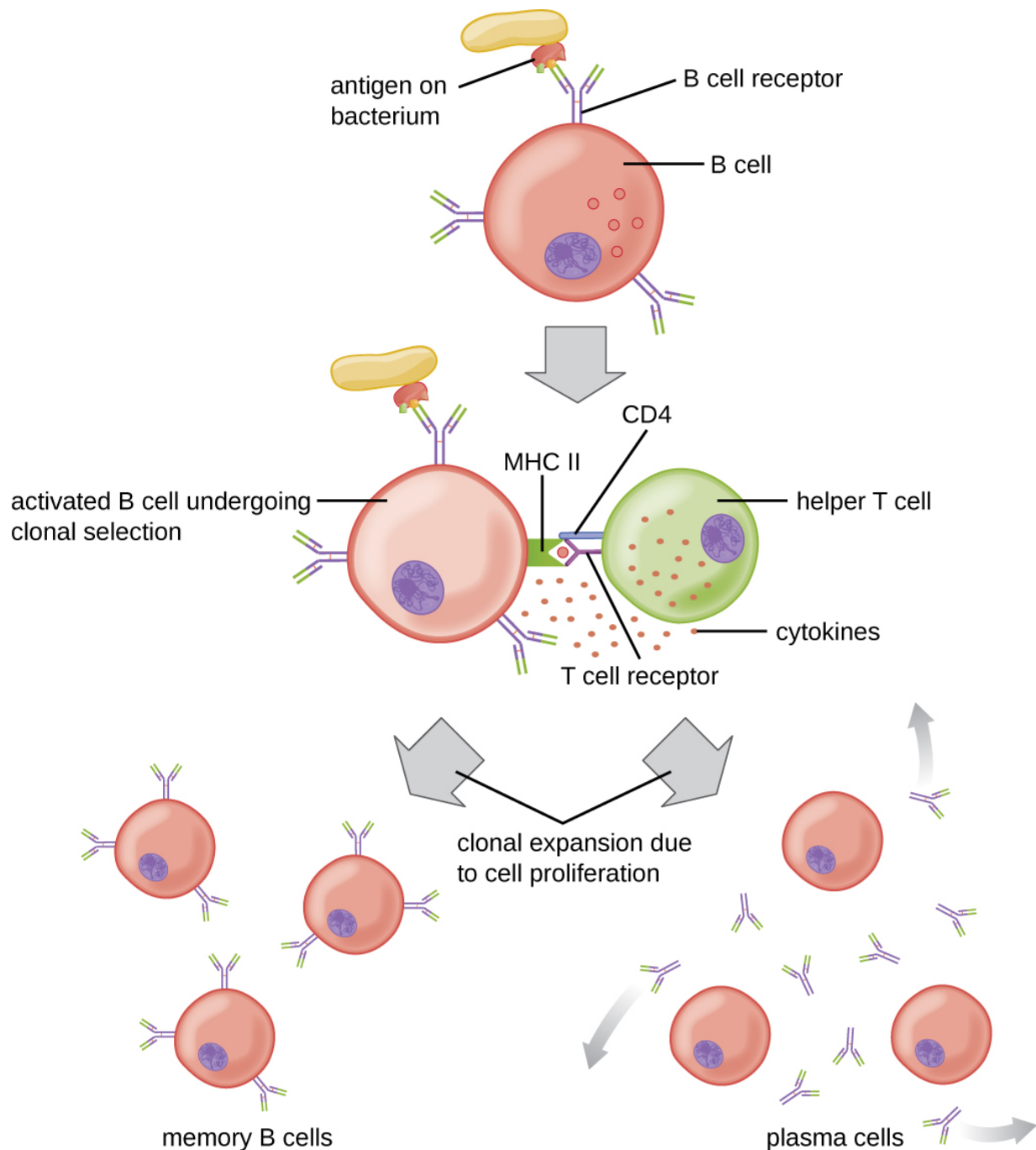
T Cell-Dependent Activation of B cells

T cell-dependent activation of B cells is more complex than T cell-independent activation, but the resulting immune response is stronger and develops memory. T cell-dependent activation can occur either in response to free protein antigens or to protein antigens associated with an intact pathogen. Interaction between the BCRs on a naïve mature B cell and a free

protein antigen stimulate internalization of the antigen, whereas interaction with antigens associated with an intact pathogen initiates the extraction of the antigen from the pathogen before internalization. Once internalized inside the B cell, the protein antigen is processed and presented with MHC II. The presented antigen is then recognized by helper T cells specific to the same antigen. The TCR of the helper T cell recognizes the foreign antigen, and the T cell's CD4 molecule interacts with MHC II on the B cell. The coordination between B cells and helper T cells that are specific to the same antigen is referred to as **linked recognition**.

Once activated by linked recognition, T_H2 cells produce and secrete cytokines that activate the B cell and cause proliferation into clonal daughter cells. After several rounds of proliferation, additional cytokines provided by the T_H2 cells stimulate the differentiation of activated B cell clones into **memory B cells**, which will quickly respond to subsequent exposures to the same protein epitope, and plasma cells that lose their membrane BCRs and initially secrete pentameric IgM ([link](#)).

After initial secretion of IgM, cytokines secreted by T_H2 cells stimulate the plasma cells to switch from IgM production to production of IgG, IgA, or IgE. This process, called **class switching** or isotype switching, allows plasma cells cloned from the same activated B cell to produce a variety of antibody classes with the same epitope specificity. Class switching is accomplished by genetic rearrangement of gene segments encoding the constant region, which determines an antibody's class. The variable region is not changed, so the new class of antibody retains the original epitope specificity.



In T cell-dependent activation of B cells, the B cell recognizes and internalizes an antigen and presents it to a helper T cell that is specific to the same antigen. The helper T cell interacts with the antigen presented by the B cell, which activates the T cell and stimulates the release of cytokines that then activate the B cell. Activation of the B

cell triggers proliferation and differentiation into B cells and plasma cells.

Note:

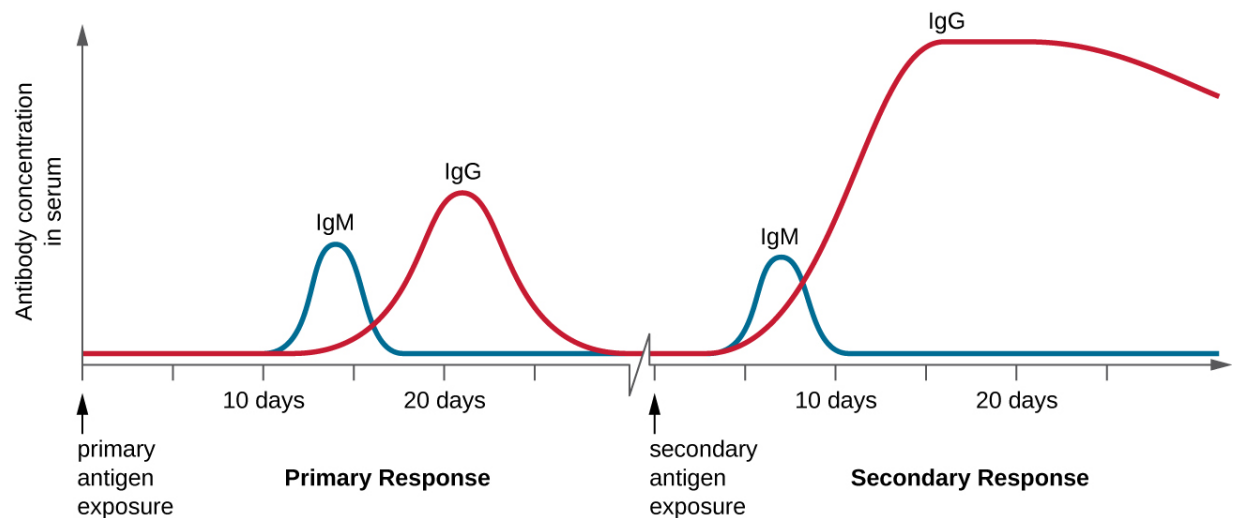
- What steps are required for T cell-dependent activation of B cells?
- What is antibody class switching and why is it important?

Primary and Secondary Responses

T cell-dependent activation of B cells plays an important role in both the primary and secondary responses associated with adaptive immunity. With the first exposure to a protein antigen, a T cell-dependent primary antibody response occurs. The initial stage of the primary response is a **lag period**, or latent period, of approximately 10 days, during which no antibody can be detected in serum. This lag period is the time required for all of the steps of the primary response, including naïve mature B cell binding of antigen with BCRs, antigen processing and presentation, helper T cell activation, B cell activation, and clonal proliferation. The end of the lag period is characterized by a rise in IgM levels in the serum, as T_H2 cells stimulate B cell differentiation into plasma cells. IgM levels reach their peak around 14 days after primary antigen exposure; at about this same time, T_H2 stimulates antibody class switching, and IgM levels in serum begin to decline. Meanwhile, levels of IgG increase until they reach a peak about three weeks into the primary response ([\[link\]](#)).

During the primary response, some of the cloned B cells are differentiated into memory B cells programmed to respond to subsequent exposures. This secondary response occurs more quickly and forcefully than the primary response. The lag period is decreased to only a few days and the production of IgG is significantly higher than observed for the primary response ([\[link\]](#)). In addition, the antibodies produced during the secondary response

are more effective and bind with higher affinity to the targeted epitopes. Plasma cells produced during secondary responses live longer than those produced during the primary response, so levels of specific antibody remain elevated for a longer period of time.



Compared to the primary response, the secondary antibody response occurs more quickly and produces antibody levels that are higher and more sustained. The secondary response mostly involves IgG.

Note:

- What events occur during the lag period of the primary antibody response?
- Why do antibody levels remain elevated longer during the secondary antibody response?

Key Concepts and Summary

- **B lymphocytes** or **B cells** produce antibodies involved in humoral immunity. B cells are produced in the bone marrow, where the initial stages of maturation occur, and travel to the spleen for final steps of maturation into naïve mature B cells.
- **B-cell receptors (BCRs)** are membrane-bound monomeric forms of IgD and IgM that bind specific antigen epitopes with their Fab antigen-binding regions. Diversity of antigen binding specificity is created by genetic rearrangement of V, D, and J segments similar to the mechanism used for TCR diversity.
- Protein antigens are called **T-dependent antigens** because they can only activate B cells with the cooperation of helper T cells. Other molecule classes do not require T cell cooperation and are called **T-independent antigens**.
- **T cell-independent activation** of B cells involves cross-linkage of BCRs by repetitive nonprotein antigen epitopes. It is characterized by the production of IgM by **plasma cells** and does not produce memory B cells.
- **T cell-dependent activation** of B cells involves processing and presentation of protein antigens to helper T cells, activation of the B cells by cytokines secreted from activated T_H2 cells, and plasma cells that produce different classes of antibodies as a result of **class switching**. **Memory B cells** are also produced.
- Secondary exposures to T-dependent antigens result in a secondary antibody response initiated by memory B cells. The secondary response develops more quickly and produces higher and more sustained levels of antibody with higher affinity for the specific antigen.

Multiple Choice

Exercise:

Problem: Which of the following would be a T-dependent antigen?

- lipopolysaccharide
- glycolipid
- protein

d. carbohydrate

Solution:

C

Exercise:

Problem: Which of the following would be a BCR?

- a. CD4
 - b. MHC II
 - c. MHC I
 - d. IgD
-

Solution:

D

Exercise:

Problem:

Which of the following does not occur during the lag period of the primary antibody response?

- a. activation of helper T cells
 - b. class switching to IgG
 - c. presentation of antigen with MHC II
 - d. binding of antigen to BCRs
-

Solution:

B

Fill in the Blank

Exercise:**Problem:**

_____ antigens can stimulate B cells to become activated but require cytokine assistance delivered by helper T cells.

Solution:

T-dependent

Exercise:**Problem:**

T-independent antigens can stimulate B cells to become activated and secrete antibodies without assistance from helper T cells. These antigens possess _____ antigenic epitopes that cross-link BCRs.

Solution:

repetitive

Critical Thinking**Exercise:****Problem:**

A patient lacks the ability to make functioning T cells because of a genetic disorder. Would this patient's B cells be able to produce antibodies in response to an infection? Explain your answer.

LEARNING OBJECTIVES

- Compare the various kinds of artificial immunity
- Differentiate between variolation and vaccination
- Describe different types of vaccines and explain their respective advantages and disadvantages

For many diseases, prevention is the best form of treatment, and few strategies for disease prevention are as effective as vaccination. Vaccination is a form of artificial immunity. By artificially stimulating the adaptive immune defenses, a vaccine triggers memory cell production similar to that which would occur during a primary response. In so doing, the patient is able to mount a strong secondary response upon exposure to the pathogen—but without having to first suffer through an initial infection. In this section, we will explore several different kinds of artificial immunity along with various types of vaccines and the mechanisms by which they induce artificial immunity.

Classifications of Adaptive Immunity

All forms of adaptive immunity can be described as either active or passive. **Active immunity** refers to the activation of an individual's own adaptive immune defenses, whereas **passive immunity** refers to the transfer of adaptive immune defenses from another individual or animal. Active and passive immunity can be further subdivided based on whether the protection is acquired naturally or artificially.

Natural active immunity is adaptive immunity that develops after natural exposure to a pathogen ([link](#)). Examples would include the lifelong immunity that develops after recovery from a chickenpox or measles infection (although an acute infection is not always necessary to activate adaptive immunity). The length of time that an individual is protected can vary substantially depending upon the pathogen and antigens involved. For example, activation of adaptive immunity by protein spike structures during an intracellular viral infection can activate lifelong immunity, whereas activation by carbohydrate capsule antigens during an extracellular bacterial infection may activate shorter-term immunity.

Natural passive immunity involves the natural passage of antibodies from a mother to her child before and after birth. IgG is the only antibody class that can cross the placenta from mother's blood to the fetal blood supply. Placental transfer of IgG is an important passive immune defense for the infant, lasting up to six months after birth. Secretory IgA can also be transferred from mother to infant through breast milk.

Artificial passive immunity refers to the transfer of antibodies produced by a donor (human or animal) to another individual. This transfer of antibodies may be done as a prophylactic measure (i.e., to prevent disease after exposure to a pathogen) or as a strategy for treating an active infection. For

example, artificial passive immunity is commonly used for post-exposure prophylaxis against rabies, hepatitis A, hepatitis B, and chickenpox (in high risk individuals). Active infections treated by artificial passive immunity include cytomegalovirus infections in immunocompromised patients and Ebola virus infections. In 1995, eight patients in the Democratic Republic of the Congo with active Ebola infections were treated with blood transfusions from patients who were recovering from Ebola. Only one of the eight patients died (a 12.5% mortality rate), which was much lower than the expected 80% mortality rate for Ebola in untreated patients.[\[footnote\]](#) Artificial passive immunity is also used for the treatment of diseases caused by bacterial toxins, including tetanus, botulism, and diphtheria. K. Mupapa, M. Massamba, K. Kibadi, K. Kivula, A. Bwaka, M. Kipasa, R. Colebunders, J. J. Muyembe-Tamfum. “Treatment of Ebola Hemorrhagic Fever with Blood Transfusions from Convalescent Patients.” *Journal of Infectious Diseases* 179 Suppl. (1999): S18–S23.

Artificial active immunity is the foundation for vaccination. It involves the activation of adaptive immunity through the deliberate exposure of an individual to weakened or inactivated pathogens, or preparations consisting of key pathogen antigens.

Mechanisms of Acquisition of Immunity		
	Natural acquired	Artificial acquired
Passive	Immunity acquired from antibodies passed in breast milk or through placenta 	Immunity gained through antibodies harvested from another person or an animal 
Active	Immunity gained through illness and recovery 	Immunity acquired through a vaccine 

The four classifications of immunity. (credit top left photo: modification of work by USDA; credit top right photo: modification of work by “Michaelberry”/Wikimedia; credit bottom left photo: modification of work by Centers for Disease Control and Prevention; credit bottom right photo: modification of work by Friskila Silitonga, Indonesia, Centers for Disease Control and Prevention)

Note:

- What is the difference between active and passive immunity?
- What kind of immunity is conferred by a vaccine?

Herd Immunity

The four kinds of immunity just described result from an individual's adaptive immune system. For any given disease, an individual may be considered immune or susceptible depending on his or her ability to mount an effective immune response upon exposure. Thus, any given population is likely to have some individuals who are immune and other individuals who are susceptible. If a population has very few susceptible individuals, even those susceptible individuals will be protected by a phenomenon called **herd immunity**. Herd immunity has nothing to do with an individual's ability to mount an effective immune response; rather, it occurs because there are too few susceptible individuals in a population for the disease to spread effectively.

Vaccination programs create herd immunity by greatly reducing the number of susceptible individuals in a population. Even if some individuals in the population are not vaccinated, as long as a certain percentage is immune (either naturally or artificially), the few susceptible individuals are unlikely to be exposed to the pathogen. However, because new individuals are constantly entering populations (for example, through birth or relocation), vaccination programs are necessary to maintain herd immunity.

Note:

Vaccination: Obligation or Choice

A growing number of parents are choosing not to vaccinate their children. They are dubbed “antivaxxers,” and the majority of them believe that vaccines are a cause of autism (or other disease conditions), a link that has now been thoroughly disproven. Others object to vaccines on religious or moral grounds (e.g., the argument that Gardasil vaccination against HPV may promote sexual promiscuity), on personal ethical grounds (e.g., a conscientious objection to any medical intervention), or on political grounds (e.g., the notion that mandatory vaccinations are a violation of individual liberties).[\[footnote\]](#)

Elizabeth Yale. “Why Anti-Vaccination Movements Can Never Be Tamed.” *Religion & Politics*, July 22, 2014. <http://religionandpolitics.org/2014/07/22/why-anti-vaccination-movements-can-never-be-tamed>.

It is believed that this growing number of unvaccinated individuals has led to new outbreaks of whooping cough and measles. We would expect that herd immunity would protect those unvaccinated in our population, but herd immunity can only be maintained if enough individuals are being vaccinated.

Vaccination is clearly beneficial for public health. But from the individual parent's perspective the view can be murkier. Vaccines, like all medical interventions, have associated risks, and while the risks of vaccination may be extremely low compared to the risks of infection, parents may not always understand or accept the consensus of the medical community. Do such parents have a right to withhold vaccination from their children? Should they be allowed to put their children—and society at large—at risk?

Many governments insist on childhood vaccinations as a condition for entering public school, but it has become easy in most states to opt out of the requirement or to keep children out of the public system. Since the 1970s, West Virginia and Mississippi have had in place a stringent requirement for childhood vaccination, without exceptions, and neither state has had a case of measles since the early 1990s. California lawmakers recently passed a similar law in response to a measles outbreak in 2015, making it much more difficult for parents to opt out of vaccines if their children are attending public schools. Given this track record and renewed legislative efforts, should other states adopt similarly strict requirements?

What role should health-care providers play in promoting or enforcing universal vaccination? Studies have shown that many parents' minds can be changed in response to information delivered by health-

care workers, but is it the place of health-care workers to try to persuade parents to have their children vaccinated? Some health-care providers are understandably reluctant to treat unvaccinated patients. Do they have the right to refuse service to patients who decline vaccines? Do insurance companies have the right to deny coverage to antivaxxers? These are all ethical questions that policymakers may be forced to address as more parents skirt vaccination norms.

Variolation and Vaccination

Thousands of years ago, it was first recognized that individuals who survived a smallpox infection were immune to subsequent infections. The practice of inoculating individuals to actively protect them from smallpox appears to have originated in the 10th century in China, when the practice of **variolation** was described ([link](#)). Variolation refers to the deliberate inoculation of individuals with infectious material from scabs or pustules of smallpox victims. Infectious materials were either injected into the skin or introduced through the nasal route. The infection that developed was usually milder than naturally acquired smallpox, and recovery from the milder infection provided protection against the more serious disease.

Although the majority of individuals treated by variolation developed only mild infections, the practice was not without risks. More serious and sometimes fatal infections did occur, and because smallpox was contagious, infections resulting from variolation could lead to epidemics. Even so, the practice of variolation for smallpox prevention spread to other regions, including India, Africa, and Europe.



Variolation for smallpox originated in the Far East and the practice later spread to Europe and Africa. This Japanese

practice later spread to Europe and Africa. This Japanese relief depicts a patient receiving a smallpox variolation from the physician Ogata Shunsaku (1748–1810).

Although variolation had been practiced for centuries, the English physician Edward Jenner (1749–1823) is generally credited with developing the modern process of vaccination. Jenner observed that milkmaids who developed cowpox, a disease similar to smallpox but milder, were immune to the more serious smallpox. This led Jenner to hypothesize that exposure to a less virulent pathogen could provide immune protection against a more virulent pathogen, providing a safer alternative to variolation. In 1796, Jenner tested his hypothesis by obtaining infectious samples from a milkmaid's active cowpox lesion and injecting the materials into a young boy ([link](#)). The boy developed a mild infection that included a low-grade fever, discomfort in his axillae (armpit) and loss of appetite. When the boy was later infected with infectious samples from smallpox lesions, he did not contract smallpox. [\[footnote\]](#) This new approach was termed **vaccination**, a name deriving from the use of cowpox (Latin *vacca* meaning “cow”) to protect against smallpox. Today, we know that Jenner's vaccine worked because the cowpox virus is genetically and antigenically related to the *Variola* viruses that caused smallpox. Exposure to cowpox antigens resulted in a primary response and the production of memory cells that identical or related epitopes of *Variola* virus upon a later exposure to smallpox. N. J. Willis. “Edward Jenner and the Eradication of Smallpox.” *Scottish Medical Journal* 42 (1997): 118–121.

The success of Jenner's smallpox vaccination led other scientists to develop vaccines for other diseases. Perhaps the most notable was Louis Pasteur, who developed vaccines for rabies, cholera, and anthrax. During the 20th and 21st centuries, effective vaccines were developed to prevent a wide range of diseases caused by viruses (e.g., chickenpox and shingles, hepatitis, measles, mumps, polio, and yellow fever) and bacteria (e.g., diphtheria, pneumococcal pneumonia, tetanus, and whooping cough,).



(a)



(b)

(a) A painting of Edward Jenner depicts a cow and a milkmaid in the background. (b) Lesions on a patient infected with cowpox, a zoonotic disease caused by a virus closely related to the one that

causes smallpox. (credit b: modification of work by the Centers for Disease Control and Prevention)

Note:

- What is the difference between variolation and vaccination for smallpox?
- Explain why vaccination is less risky than variolation.

Classes of Vaccines

For a vaccine to provide protection against a disease, it must expose an individual to pathogen-specific antigens that will stimulate a protective adaptive immune response. By its very nature, this entails some risk. As with any pharmaceutical drug, vaccines have the potential to cause adverse effects. However, the ideal vaccine causes no severe adverse effects and poses no risk of contracting the disease that it is intended to prevent. Various types of vaccines have been developed with these goals in mind. These different classes of vaccines are described in the next section and summarized in [\[link\]](#).

Live Attenuated Vaccines

Live attenuated vaccines expose an individual to a weakened strain of a pathogen with the goal of establishing a subclinical infection that will activate the adaptive immune defenses. Pathogens are attenuated to decrease their virulence using methods such as genetic manipulation (to eliminate key virulence factors) or long-term culturing in an unnatural host or environment (to promote mutations and decrease virulence).

By establishing an active infection, live attenuated vaccines stimulate a more comprehensive immune response than some other types of vaccines. Live attenuated vaccines activate both cellular and humoral immunity and stimulate the development of memory for long-lasting immunity. In some cases, vaccination of one individual with a live attenuated pathogen can even lead to natural transmission of the attenuated pathogen to other individuals. This can cause the other individuals to also develop an active, subclinical infection that activates their adaptive immune defenses.

Disadvantages associated with live attenuated vaccines include the challenges associated with long-term storage and transport as well as the potential for a patient to develop signs and symptoms of disease during the active infection (particularly in immunocompromised patients). There is also a risk of the attenuated pathogen reverting back to full virulence. [\[link\]](#) lists examples live attenuated vaccines.

Inactivated Vaccines

Inactivated vaccines contain whole pathogens that have been killed or inactivated with heat, chemicals, or radiation. For inactivated vaccines to be effective, the inactivation process must not affect the structure of key antigens on the pathogen.

Because the pathogen is killed or inactive, inactivated vaccines do not produce an active infection, and the resulting immune response is weaker and less comprehensive than that provoked by a live attenuated vaccine. Typically the response involves only humoral immunity, and the pathogen cannot be transmitted to other individuals. In addition, inactivated vaccines usually require higher doses and multiple boosters, possibly causing inflammatory reactions at the site of injection.

Despite these disadvantages, inactivated vaccines do have the advantages of long-term storage stability and ease of transport. Also, there is no risk of causing severe active infections. However, inactivated vaccines are not without their side effects. [\[link\]](#) lists examples of inactivated vaccines.

Subunit Vaccines

Whereas live attenuated and inactive vaccines expose an individual to a weakened or dead pathogen, **subunit vaccines** only expose the patient to the key antigens of a pathogen—not whole cells or viruses. Subunit vaccines can be produced either by chemically degrading a pathogen and isolating its key antigens or by producing the antigens through genetic engineering. Because these vaccines contain only the essential antigens of a pathogen, the risk of side effects is relatively low. [\[link\]](#) lists examples of subunit vaccines.

Toxoid Vaccines

Like subunit vaccines, **toxoid vaccines** do not introduce a whole pathogen to the patient; they contain inactivated bacterial toxins, called toxoids. Toxoid vaccines are used to prevent diseases in which bacterial toxins play an important role in pathogenesis. These vaccines activate humoral immunity that neutralizes the toxins. [\[link\]](#) lists examples of toxoid vaccines.

Conjugate Vaccines

A **conjugate vaccine** is a type of subunit vaccine that consists of a protein conjugated to a capsule polysaccharide. Conjugate vaccines have been developed to enhance the efficacy of subunit vaccines against pathogens that have protective polysaccharide capsules that help them evade phagocytosis, causing invasive infections that can lead to meningitis and other serious conditions. The subunit vaccines against these pathogens introduce T-independent capsular polysaccharide antigens that result in the production of antibodies that can opsonize the capsule and thus combat the infection; however, children under the age of two years do not respond effectively to these vaccines. Children do respond effectively when vaccinated with the conjugate vaccine, in which a protein with T-dependent antigens is conjugated to the capsule polysaccharide. The conjugated protein-polysaccharide antigen stimulates production of antibodies against both the protein and the capsule polysaccharide. [\[link\]](#) lists examples of conjugate vaccines.

Classes of Vaccines				
Class	Description	Advantages	Disadvantages	Examples
Live attenuated	Weakened strain of whole pathogen	Cellular and humoral immunity	Difficult to store and transport	Chickenpox, German measles, measles, mumps, tuberculosis, typhoid fever, yellow fever
		Long-lasting immunity	Risk of infection in immunocompromised patients	
		Transmission to contacts	Risk of reversion	
Inactivated	Whole pathogen killed or inactivated with heat, chemicals, or radiation	Ease of storage and transport	Weaker immunity (humoral only)	Cholera, hepatitis A, influenza, plague, rabies
		No risk of severe active infection	Higher doses and more boosters required	
Subunit	Immunogenic antigens	Lower risk of side effects	Limited longevity	Anthrax, hepatitis B, influenza, meningitis, papillomavirus, pneumococcal pneumonia, whooping cough
			Multiple doses required	
			No protection against antigenic variation	
Toxoid	Inactivated bacterial toxin	Humoral immunity to neutralize toxin	Does not prevent infection	Botulism, diphtheria, pertussis, tetanus
Conjugate	Capsule polysaccharide conjugated to protein	T-dependent response to capsule	Costly to produce	Meningitis (<i>Haemophilus influenzae</i> , <i>Streptococcus pneumoniae</i> , <i>Neisseria meningitides</i>)
		Better response in young children	No protection against antigenic variation	
			May interfere with other vaccines	

Note:

- What is the risk associated with a live attenuated vaccine?
- Why is a conjugated vaccine necessary in some cases?

Note:**DNA Vaccines**

DNA vaccines represent a relatively new and promising approach to vaccination. A DNA vaccine is produced by incorporating genes for antigens into a recombinant plasmid vaccine. Introduction of the DNA vaccine into a patient leads to uptake of the recombinant plasmid by some of the patient's cells, followed by transcription and translation of antigens and presentation of these antigens with MHC I to activate adaptive immunity. This results in the stimulation of both humoral and cellular immunity without the risk of active disease associated with live attenuated vaccines.

Although most DNA vaccines for humans are still in development, it is likely that they will become more prevalent in the near future as researchers are working on engineering DNA vaccines that will activate adaptive immunity against several different pathogens at once. First-generation DNA vaccines tested in the 1990s looked promising in animal models but were disappointing when tested in human subjects. Poor cellular uptake of the DNA plasmids was one of the major problems impacting their efficacy. Trials of second-generation DNA vaccines have been more promising thanks to new techniques for enhancing cellular uptake and optimizing antigens. DNA vaccines for various cancers and viral pathogens such as HIV, HPV, and hepatitis B and C are currently in development.

Some DNA vaccines are already in use. In 2005, a DNA vaccine against West Nile virus was approved for use in horses in the United States. Canada has also approved a DNA vaccine to protect fish from infectious hematopoietic necrosis virus.[\[footnote\]](#) A DNA vaccine against Japanese encephalitis virus was approved for use in humans in 2010 in Australia.[\[footnote\]](#)

M. Alonso and J. C. Leong. "Licensed DNA Vaccines Against Infectious Hematopoietic Necrosis Virus (IHNV)." *Recent Patents on DNA & Gene Sequences (Discontinued)* 7 no. 1 (2013): 62–65, issn 1872-2156/2212-3431. doi 10.2174/1872215611307010009.

S.B. Halstead and S. J. Thomas. "New Japanese Encephalitis Vaccines: Alternatives to Production in Mouse Brain." *Expert Review of Vaccines* 10 no. 3 (2011): 355–64.

Note:**Resolution**

Based on Olivia's symptoms, her physician made a preliminary diagnosis of bacterial meningitis without waiting for positive identification from the blood and CSF samples sent to the lab. Olivia was admitted to the hospital and treated with intravenous broad-spectrum antibiotics and rehydration therapy. Over the next several days, her condition began to improve, and new blood samples and lumbar puncture samples showed an absence of microbes in the blood and CSF with levels of white blood cells returning to normal. During this time, the lab produced a positive identification of *Neisseria meningitidis*, the causative agent of meningococcal meningitis, in her original CSF sample. *N. meningitidis* produces a polysaccharide capsule that serves as a virulence factor. *N. meningitidis* tends to affect infants after they begin to lose the natural passive immunity provided by maternal antibodies. At one year of age, Olivia's maternal IgG antibodies would have disappeared, and she would not have developed memory cells capable of recognizing antigens associated with the polysaccharide capsule of the *N. meningitidis*. As a result, her adaptive immune system was unable to produce protective antibodies to combat the infection, and without antibiotics she may not have

survived. Olivia's infection likely would have been avoided altogether had she been vaccinated. A conjugate vaccine to prevent meningococcal meningitis is available and approved for infants as young as two months of age. However, current vaccination schedules in the United States recommend that the vaccine be administered at age 11–12 with a booster at age 16.

Go back to the [previous Clinical Focus box](#).

Note:

In countries with developed public health systems, many vaccines are routinely administered to children and adults. Vaccine schedules are changed periodically, based on new information and research results gathered by public health agencies. In the United States, the CDC publishes [schedules and other updated information](#) about vaccines.

Key Concepts and Summary

- Adaptive immunity can be divided into four distinct classifications: **natural active immunity**, **natural passive immunity**, **artificial passive immunity**, and **artificial active immunity**.
- Artificial active immunity is the foundation for **vaccination** and vaccine development. Vaccination programs not only confer artificial immunity on individuals, but also foster **herd immunity** in populations.
- **Variolation** against smallpox originated in the 10th century in China, but the procedure was risky because it could cause the disease it was intended to prevent. Modern vaccination was developed by Edward Jenner, who developed the practice of inoculating patients with infectious materials from cowpox lesions to prevent smallpox.
- **Live attenuated vaccines** and **inactivated vaccines** contain whole pathogens that are weak, killed, or inactivated. **Subunit vaccines**, **toxoid vaccines**, and **conjugate vaccines** contain acellular components with antigens that stimulate an immune response.

Multiple Choice

Exercise:

Problem:

A patient is bitten by a dog with confirmed rabies infection. After treating the bite wound, the physician injects the patient with antibodies that are specific for the rabies virus to prevent the development of an active infection. This is an example of:

- a. Natural active immunity
- b. Artificial active immunity
- c. Natural passive immunity
- d. Artificial passive immunity

Solution:

D

Exercise:

Problem:

A patient gets a cold, and recovers a few days later. The patient's classmates come down with the same cold roughly a week later, but the original patient does not get the same cold again. This is an example of:

- a. Natural active immunity
- b. Artificial active immunity
- c. Natural passive immunity
- d. Artificial passive immunity

Solution:

A

Matching**Exercise:**

Problem: Match each type of vaccine with the corresponding example.

___inactivated vaccine	A. Weakened influenza virions that can only replicate in the slightly lower temperatures of the nasal passages are sprayed into the nose. They do not cause serious flu symptoms, but still produce an active infection that induces a protective adaptive immune response.
___live attenuated vaccine	B. Tetanus toxin molecules are harvested and chemically treated to render them harmless. They are then injected into a patient's arm.
___toxoid vaccine	C. Influenza virus particles grown in chicken eggs are harvested and chemically treated to render them noninfectious. These immunogenic particles are then purified and packaged and administered as an injection.
___subunit vaccine	D. The gene for hepatitis B virus surface antigen is inserted into a yeast genome. The modified yeast is grown and the virus protein is produced, harvested, purified, and used in a vaccine.

Solution:

C, A, B, D

Fill in the Blank

Exercise:

Problem:

A(n) _____ pathogen is in a weakened state; it is still capable of stimulating an immune response but does not cause a disease.

Solution:

attenuated

Exercise:

Problem:

_____ immunity occurs when antibodies from one individual are harvested and given to another to protect against disease or treat active disease.

Solution:

Artificial passive

Exercise:

Problem:

In the practice of _____, scabs from smallpox victims were used to immunize susceptible individuals against smallpox.

Solution:

variola

Short answer

Exercise:

Problem: Briefly compare the pros and cons of inactivated versus live attenuated vaccines.

Introduction

class="introduction"

Bee stings
and other
allergens
can cause
life-
threatening,
systemic
allergic
reactions.
Sensitive
individuals
may need to
carry an
epinephrine
auto-
injector
(e.g.,
EpiPen) in
case of a
sting. A
bee-sting
allergy is an
example of
an immune
response
that is
harmful to
the host
rather than
protective;
epinephrine
counteracts
the severe
drop in

blood
pressure that
can result
from the
immune
response.
(credit right:
modification
of work
by Carol
Bleistine)



An allergic reaction is an immune response to a type of antigen called an allergen. Allergens can be found in many different items, from peanuts and insect stings to latex and some drugs. Unlike other kinds of antigens, allergens are not necessarily associated with pathogenic microbes, and many allergens provoke no immune response at all in most people.

Allergic responses vary in severity. Some are mild and localized, like hay fever or hives, but others can result in systemic, life-threatening reactions. Anaphylaxis, for example, is a rapidly developing allergic reaction that can cause a dangerous drop in blood pressure and severe swelling of the throat that may close off the airway.

Allergies are just one example of how the immune system—the system normally responsible for preventing disease—can actually cause or mediate disease symptoms. In this chapter, we will further explore allergies and other disorders of the immune system, including hypersensitivity reactions, autoimmune diseases, transplant rejection, and diseases associated with immunodeficiency.

Hypersensitivities

LEARNING OBJECTIVES

- Identify and compare the distinguishing characteristics, mechanisms, and major examples of type I, II, III, and IV hypersensitivities

Note:

Part 1

Kerry, a 40-year-old airline pilot, has made an appointment with her primary care physician to discuss a rash that develops whenever she spends time in the sun. As she explains to her physician, it does not seem like sunburn. She is careful not to spend too much time in the sun and she uses sunscreen. Despite these precautions, the rash still appears, manifesting as red, raised patches that get slightly scaly. The rash persists for 7 to 10 days each time, and it seems to largely go away on its own. Lately, the rashes have also begun to appear on her cheeks and above her eyes on either side of her forehead.

- Is Kerry right to be concerned, or should she simply be more careful about sun exposure?
- Are there conditions that might be brought on by sun exposure that Kerry's physician should be considering?

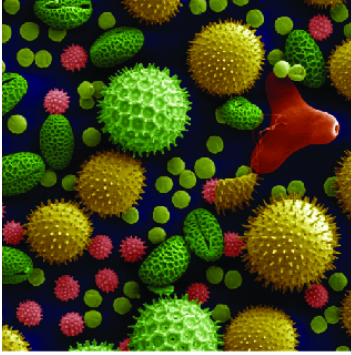
Jump to the [next](#) Clinical Focus box.

In [Adaptive Specific Host Defenses](#), we discussed the mechanisms by which adaptive immune defenses, both humoral and cellular, protect us from infectious diseases. However, these same protective immune defenses can also be responsible for undesirable reactions called **hypersensitivity** reactions. Hypersensitivity reactions are classified by their immune mechanism.

- Type I hypersensitivity reactions involve immunoglobulin E (IgE) antibody against soluble antigen, triggering mast cell degranulation.
- Type II hypersensitivity reactions involve IgG and IgM antibodies directed against cellular antigens, leading to cell damage mediated by other immune system effectors.
- Type III hypersensitivity reactions involve the interactions of IgG, IgM, and, occasionally, IgA[\[footnote\]](#) antibodies with antigen to form immune complexes. Accumulation of immune complexes in tissue leads to tissue damage mediated by other immune system effectors. D.S. Strayer et al (eds). *Rubin's Pathology: Clinicopathologic Foundations of Medicine*. 7th ed. Philadelphia, PA: Lippincott, Williams & Wilkins, 2014.
- Type IV hypersensitivity reactions are T-cell-mediated reactions that can involve tissue damage mediated by activated macrophages and cytotoxic T cells.

Type I Hypersensitivities

When a presensitized individual is exposed to an **allergen**, it can lead to a rapid immune response that occurs almost immediately. Such a response is called an **allergy** and is classified as a **type I hypersensitivity**. Allergens may be seemingly harmless substances such as animal dander, molds, or pollen. Allergens may also be substances considered innately more hazardous, such as insect venom or therapeutic drugs. Food intolerances can also yield allergic reactions as individuals become sensitized to foods such as peanuts or shellfish ([\[link\]](#)). Regardless of the allergen, the first exposure activates a primary IgE antibody response that sensitizes an individual to type I hypersensitivity reaction upon subsequent exposure.



(a)



(b)



(c)

(a) Allergens in plant pollen, shown here in a colorized electron micrograph, may trigger allergic rhinitis or hay fever in sensitive individuals. (b) Skin rashes are often associated with allergic reactions. (c) Peanuts can be eaten safely by most people but can provoke severe allergic reactions in sensitive individuals.

For susceptible individuals, a first exposure to an allergen activates a strong T_H2 cell response ([\[link\]](#)). Cytokines interleukin (IL)-4 and IL-13 from the T_H2 cells activate B cells specific to the same allergen, resulting in clonal proliferation, differentiation into plasma cells, and antibody-class switch from production of IgM to production of IgE. The fragment crystallizable (Fc) regions of the IgE antibodies bind to specific receptors on the surface of mast cells throughout the body. It is estimated that each mast cell can bind up to 500,000 IgE molecules, with each IgE molecule having two allergen-specific fragment antigen-binding (Fab) sites available for binding allergen on subsequent exposures. By the time this occurs, the allergen is often no longer present and there is no allergic reaction, but the mast cells are primed for a subsequent exposure and the individual is sensitized to the allergen.

On subsequent exposure, allergens bind to multiple IgE molecules on mast cells, cross-linking the IgE molecules. Within minutes, this cross-linking of IgE activates the mast cells and triggers **degranulation**, a reaction in which the contents of the granules in the mast cell are released into the extracellular environment. Preformed components that are released from

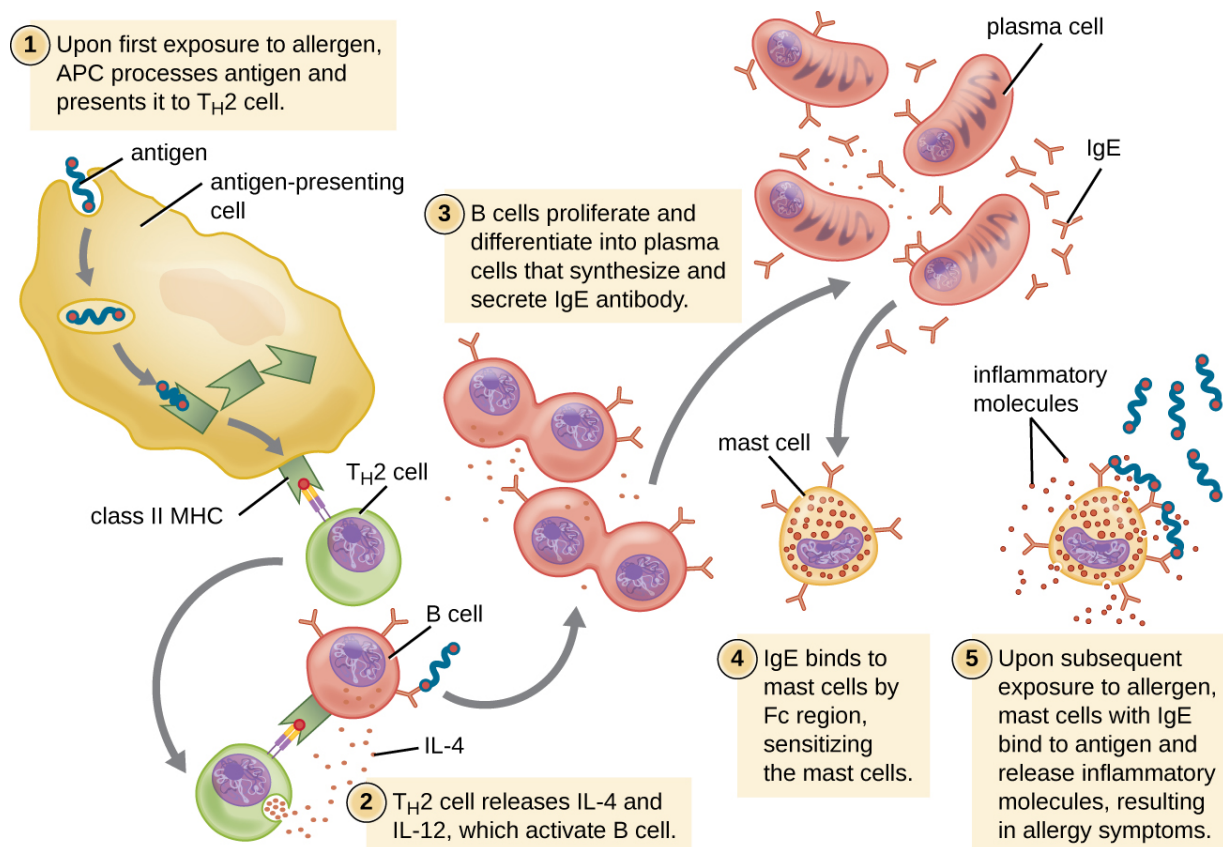
granules include histamine, serotonin, and bradykinin ([link](#)). The activated mast cells also release newly formed lipid mediators (leukotrienes and prostaglandins from membrane arachadonic acid metabolism) and cytokines such as tumor necrosis factor ([link](#)).

The chemical mediators released by mast cells collectively cause the inflammation and signs and symptoms associated with type I hypersensitivity reactions. Histamine stimulates mucus secretion in nasal passages and tear formation from lacrimal glands, promoting the runny nose and watery eyes of allergies. Interaction of histamine with nerve endings causes itching and sneezing. The vasodilation caused by several of the mediators can result in hives, headaches, angioedema (swelling that often affects the lips, throat, and tongue), and hypotension (low blood pressure). Bronchiole constriction caused by some of the chemical mediators leads to wheezing, dyspnea (difficulty breathing), coughing, and, in more severe cases, cyanosis (bluish color to the skin or mucous membranes). Vomiting can result from stimulation of the vomiting center in the cerebellum by histamine and serotonin. Histamine can also cause relaxation of intestinal smooth muscles and diarrhea.

Selected Preformed Components of Mast Cell Granules	
Granule Component	Activity
Heparin	Stimulates the generation of bradykinin, which causes increased vascular permeability, vasodilation, bronchiole constriction, and increased mucus secretion

Selected Preformed Components of Mast Cell Granules	
Granule Component	Activity
Histamine	Causes smooth-muscle contraction, increases vascular permeability, increases mucus and tear formation
Serotonin	Increases vascular permeability, causes vasodilation and smooth-muscle contraction

Selected Newly Formed Chemical Mediators of Inflammation and Allergic Response	
Chemical Mediator	Activity
Leukotriene	Causes smooth-muscle contraction and mucus secretion, increases vascular permeability
Prostaglandin	Causes smooth-muscle contraction and vasodilation
TNF- α (cytokine)	Causes inflammation and stimulates cytokine production by other cell types



On first exposure to an allergen in a susceptible individual, antigen-presenting cells process and present allergen epitopes with major histocompatibility complex (MHC) II to T helper cells. B cells also process and present the same allergen epitope to T_H2 cells, which release cytokines IL-4 and IL-13 to stimulate proliferation and differentiation into IgE-secreting plasma cells. The IgE molecules bind to mast cells with their Fc region, sensitizing the mast cells for activation with subsequent exposure to the allergen. With each subsequent exposure, the allergen cross-links IgE molecules on the mast cells, activating the mast cells and causing the release of preformed chemical mediators from granules (degranulation), as well as newly formed chemical mediators that collectively cause the signs and symptoms of type I hypersensitivity reactions.

Type I hypersensitivity reactions can be either localized or systemic. Localized type I hypersensitivity reactions include hay fever rhinitis, hives,

and asthma ([link](#)). Systemic type I hypersensitivity reactions are referred to as **anaphylaxis** or **anaphylactic shock**. Although anaphylaxis shares many symptoms common with the localized type I hypersensitivity reactions, the swelling of the tongue and trachea, blockage of airways, dangerous drop in blood pressure, and development of shock can make anaphylaxis especially severe and life-threatening. In fact, death can occur within minutes of onset of signs and symptoms.

Late-phase reactions in type I hypersensitivities may develop 4–12 hours after the early phase and are mediated by eosinophils, neutrophils, and lymphocytes that have been recruited by chemotactic factors released from mast cells. Activation of these recruited cells leads to the release of more chemical mediators that cause tissue damage and late-phase symptoms of swelling and redness of the skin, coughing, wheezing, and nasal discharge.

Individuals who possess genes for maladaptive traits, such as intense type I hypersensitivity reactions to otherwise harmless components of the environment, would be expected to suffer reduced reproductive success. With this kind of evolutionary selective pressure, such traits would not be expected to persist in a population. This suggests that type I hypersensitivities may have an adaptive function. There is evidence that the IgE produced during type I hypersensitivity reactions is actually meant to counter helminth infections [\[footnote\]](#). Helminths are one of few organisms that possess proteins that are targeted by IgE. In addition, there is evidence that helminth infections at a young age reduce the likelihood of type I hypersensitivities to innocuous substances later in life. Thus it may be that allergies are an unfortunate consequence of strong selection in the mammalian lineage or earlier for a defense against parasitic worms.

C.M. Fitzsimmons et al. “Helminth Allergens, Parasite-Specific IgE, and Its Protective Role in Human Immunity.” *Frontier in Immunology* 5 (2015):47.

Type I Hypersensitivities

Type I Hypersensitivities		
Type I Hypersensitivities	Cause	Signs and Symptoms
Common Name	Cause	Signs and Symptoms
Allergy-induced asthma	Inhalation of allergens	Constriction of bronchi, labored breathing, coughing, chills, body aches
Anaphylaxis	Systemic reaction to allergens	Hives, itching, swelling of tongue and throat, nausea, vomiting, low blood pressure, shock
Hay fever	Inhalation of mold or pollen	Runny nose, watery eyes, sneezing
Hives (urticaria)	Food or drug allergens, insect stings	Raised, bumpy skin rash with itching; bumps may converge into large raised areas

Note:

- What are the cells that cause a type I hypersensitivity reaction?
- Describe the differences between immediate and late-phase type I hypersensitivity reactions.
- List the signs and symptoms of anaphylaxis.

Note:

The Hygiene Hypothesis

In most modern societies, good hygiene is associated with regular bathing, and good health with cleanliness. But some recent studies suggest that the association between health and clean living may be a faulty one. Some go so far as to suggest that children should be encouraged to play in the dirt—or even eat dirt[\[footnote\]](#)—for the benefit of their health. This recommendation is based on the so-called hygiene hypothesis, which proposes that childhood exposure to antigens from a diverse range of microbes leads to a better-functioning immune system later in life.

S.T. Weiss. “Eat Dirt—The Hygiene Hypothesis and Allergic Diseases.” *New England Journal of Medicine* 347 no. 12 (2002):930–931.

The hygiene hypothesis was first suggested in 1989 by David Strachan[\[footnote\]](#), who observed an inverse relationship between the number of older children in a family and the incidence of hay fever. Although hay fever in children had increased dramatically during the mid-20th century, incidence was significantly lower in families with more children. Strachan proposed that the lower incidence of allergies in large families could be linked to infections acquired from older siblings, suggesting that these infections made children less susceptible to allergies. Strachan also argued that trends toward smaller families and a greater emphasis on cleanliness in the 20th century had decreased exposure to pathogens and thus led to higher overall rates of allergies, asthma, and other immune disorders.

D.P. Strachan “Hay Fever, Hygiene, and Household Size.” *British Medical Journal* 299 no. 6710 (1989):1259.

Other researchers have observed an inverse relationship between the incidence of immune disorders and infectious diseases that are now rare in industrialized countries but still common in less industrialized countries. [\[footnote\]](#) In developed nations, children under the age of 5 years are not exposed to many of the microbes, molecules, and antigens they almost certainly would have encountered a century ago. The lack of early challenges to the immune system by organisms with which humans and their ancestors evolved may result in failures in immune system functioning later in life.

H. Okada et al. “The ‘Hygiene Hypothesis’ for Autoimmune and Allergic Diseases: An Update.” *Clinical & Experimental Immunology* 160 no. 1 (2010):1–9.

Type II (Cytotoxic) Hypersensitivities

Immune reactions categorized as **type II hypersensitivities**, or cytotoxic hypersensitivities, are mediated by IgG and IgM antibodies binding to cell-surface antigens or matrix-associated antigens on basement membranes. These antibodies can either activate complement, resulting in an inflammatory response and lysis of the targeted cells, or they can be involved in antibody-dependent cell-mediated cytotoxicity (ADCC) with cytotoxic T cells.

In some cases, the antigen may be a self-antigen, in which case the reaction would also be described as an autoimmune disease. (Autoimmune diseases are described in [Autoimmune Disorders](#)). In other cases, antibodies may bind to naturally occurring, but exogenous, cell-surface molecules such as antigens associated with blood typing found on red blood cells (RBCs). This leads to the coating of the RBCs by antibodies, activation of the complement cascade, and complement-mediated lysis of RBCs, as well as opsonization of RBCs for phagocytosis. Two examples of type II hypersensitivity reactions involving RBCs are hemolytic transfusion reaction (HTR) and hemolytic disease of the newborn (HDN). These type II hypersensitivity reactions, which will be discussed in greater detail, are summarized in [\[link\]](#).

Immunohematology is the study of blood and blood-forming tissue in relation to the immune response. Antibody-initiated responses against blood cells are type II hypersensitivities, thus falling into the field of immunohematology. For students first learning about immunohematology, understanding the immunological mechanisms involved is made even more challenging by the complex nomenclature system used to identify different blood-group antigens, often called blood types. The first blood-group antigens either used alphabetical names or were named for the first person known to produce antibodies to the red blood cell antigen (e.g., Kell, Duffy, or Diego). However, in 1980, the International Society of Blood Transfusion (ISBT) Working Party on Terminology created a standard for blood-group terminology in an attempt to more consistently identify newly discovered blood group antigens. New antigens are now given a number

and assigned to a blood-group system, collection, or series. However, even with this effort, blood-group nomenclature is still inconsistent.

Common Type II Hypersensitivities		
Common Name	Cause	Signs and Symptoms
Hemolytic disease of the newborn (HDN)	IgG from mother crosses the placenta, targeting the fetus' RBCs for destruction	Anemia, edema, enlarged liver or spleen, hydrops (fluid in body cavity), leading to death of newborn in severe cases
Hemolytic transfusion reactions (HTR)	IgG and IgM bind to antigens on transfused RBCs, targeting donor RBCs for destruction	Fever, jaundice, hypotension, disseminated intravascular coagulation, possibly leading to kidney failure and death

ABO Blood Group Incompatibility

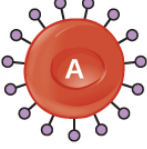
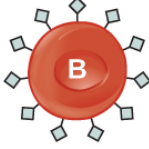
The recognition that individuals have different blood types was first described by Karl Landsteiner (1868–1943) in the early 1900s, based on his observation that serum from one person could cause a clumping of RBCs from another. These studies led Landsteiner to the identification of four distinct blood types. Subsequent research by other scientists determined that the four blood types were based on the presence or absence of surface carbohydrates “A” and “B,” and this provided the foundation for the **ABO**

blood group system that is still in use today ([\[link\]](#)). The functions of these antigens are unknown, but some have been associated with normal biochemical functions of the cell. Furthermore, ABO blood types are inherited as alleles (one from each parent), and they display patterns of dominant and codominant inheritance. The alleles for A and B blood types are codominant to each other, and both are dominant over blood type O. Therefore, individuals with genotypes of AA or AO have type A blood and express the A carbohydrate antigen on the surface of their RBCs. People with genotypes of BB or BO have type B blood and express the B carbohydrate antigen on the surface of their RBCs. Those with a genotype of AB have type AB blood and express both A and B carbohydrate antigens on the surface of their RBCs. Finally, individuals with a genotype of OO have type O blood and lack A and B carbohydrate on the surface of their RBCs.

It is important to note that the RBCs of all four ABO blood types share a common protein receptor molecule, and it is the addition of specific carbohydrates to the protein receptors that determines A, B, and AB blood types. The genes that are inherited for the A, B, and AB blood types encode enzymes that add the carbohydrate component to the protein receptor. Individuals with O blood type still have the protein receptor but lack the enzymes that would add carbohydrates that would make their red blood cell type A, B, or AB.

IgM antibodies in plasma that cross-react with blood group antigens not present on an individual's own RBCs are called **isoheamagglutinins** ([\[link\]](#)). Isoheamagglutinins are produced within the first few weeks after birth and persist throughout life. These antibodies are produced in response to exposure to environmental antigens from food and microorganisms. A person with type A blood has A antigens on the surface of their RBCs and will produce anti-B antibodies to environmental antigens that resemble the carbohydrate component of B antigens. A person with type B blood has B antigens on the surface of their RBCs and will produce anti-A antibodies to environmental antigens that are similar to the carbohydrate component of A antigens. People with blood type O lack both A and B antigens on their RBCs and, therefore, produce both anti-A and anti-B antibodies.

Conversely, people with AB blood type have both A and B antigens on their RBCs and, therefore, lack anti-A and anti-B antibodies.

	Blood Type			
	A	B	AB	O
Red blood cell type				
Isohemagglutinins	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens on red blood cell	 A antigen	 B antigen	 A and B antigens	None

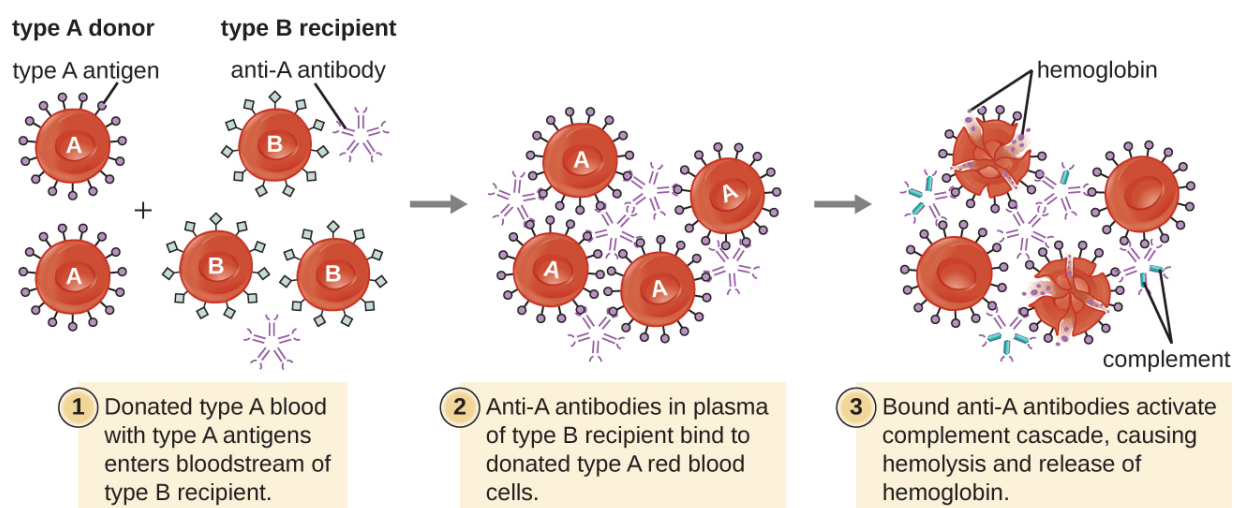
A patient may require a blood transfusion because they lack sufficient RBCs (anemia) or because they have experienced significant loss of blood volume through trauma or disease. Although the blood transfusion is given to help the patient, it is essential that the patient receive a transfusion with matching ABO blood type. A transfusion with an incompatible ABO blood type may lead to a strong, potentially lethal type II hypersensitivity cytotoxic response called **hemolytic transfusion reaction (HTR)** ([\[link\]](#)).

For instance, if a person with type B blood receives a transfusion of type A blood, their anti-A antibodies will bind to and agglutinate the transfused RBCs. In addition, activation of the classical complement cascade will lead to a strong inflammatory response, and the complement membrane attack complex (MAC) will mediate massive hemolysis of the transfused RBCs. The debris from damaged and destroyed RBCs can occlude blood vessels in the alveoli of the lungs and the glomeruli of the kidneys. Within 1 to 24

hours of an incompatible transfusion, the patient experiences fever, chills, pruritus (itching), urticaria (hives), dyspnea, hemoglobinuria (hemoglobin in the urine), and hypotension (low blood pressure). In the most serious reactions, dangerously low blood pressure can lead to shock, multi-organ failure, and death of the patient.

Hospitals, medical centers, and associated clinical laboratories typically use hemovigilance systems to minimize the risk of HTRs due to clerical error. Hemovigilance systems are procedures that track transfusion information from the donor source and blood products obtained to the follow-up of recipient patients. Hemovigilance systems used in many countries identify HTRs and their outcomes through mandatory reporting (e.g., to the Food and Drug Administration in the United States), and this information is valuable to help prevent such occurrences in the future. For example, if an HTR is found to be the result of laboratory or clerical error, additional blood products collected from the donor at that time can be located and labeled correctly to avoid additional HTRs. As a result of these measures, HTR-associated deaths in the United States occur in about one per 2 million transfused units.[\[footnote\]](#)

E.C. Vamvakas, M.A. Blajchman. “Transfusion-Related Mortality: The Ongoing Risks of Allogeneic Blood Transfusion and the Available Strategies for Their Prevention.” *Blood* 113 no. 15 (2009):3406–3417.



A type II hypersensitivity hemolytic transfusion reaction (HTR)

leading to hemolytic anemia. Blood from a type A donor is administered to a patient with type B blood. The anti-A isohemagglutinin IgM antibodies in the recipient bind to and agglutinate the incoming donor type A red blood cells. The bound anti-A antibodies activate the classical complement cascade, resulting in destruction of the donor red blood cells.

Rh Factors

Many different types of erythrocyte antigens have been discovered since the description of the ABO red cell antigens. The second most frequently described RBC antigens are **Rh factors**, named after the rhesus macaque (*Macaca mulatta*) factors identified by Karl Landsteiner and Alexander Weiner in 1940. The Rh system of RBC antigens is the most complex and immunogenic blood group system, with more than 50 specificities identified to date. Of all the Rh antigens, the one designated Rho (Weiner) or D (Fisher-Race) is the most immunogenic. Cells are classified as Rh positive (Rh+) if the Rho/D antigen is present or as Rh negative (Rh-) if the Rho/D antigen is absent. In contrast to the carbohydrate molecules that distinguish the ABO blood groups and are the targets of IgM isohemagglutinins in HTRs, the Rh factor antigens are proteins. As discussed in [B Lymphocytes and Humoral Immunity](#), protein antigens activate B cells and antibody production through a T-cell-dependent mechanism, and the T_H2 cells stimulate class switching from IgM to other antibody classes. In the case of Rh factor antigens, T_H2 cells stimulate class switching to IgG, and this has important implications for the mechanism of HDN.

Like ABO incompatibilities, blood transfusions from a donor with the wrong Rh factor antigens can cause a type II hypersensitivity HTR. However, in contrast to the IgM isohemagglutinins produced early in life through exposure to environmental antigens, production of anti-Rh factor antibodies requires the exposure of an individual with Rh- blood to Rh+ positive RBCs and activation of a primary antibody response. Although this

primary antibody response can cause an HTR in the transfusion patient, the hemolytic reaction would be delayed up to 2 weeks during the extended lag period of a primary antibody response ([B Lymphocytes and Humoral Immunity](#)). However, if the patient receives a subsequent transfusion with Rh⁺ RBCs, a more rapid HTR would occur with anti-Rh factor antibody already present in the blood. Furthermore, the rapid secondary antibody response would provide even more anti-Rh factor antibodies for the HTR.

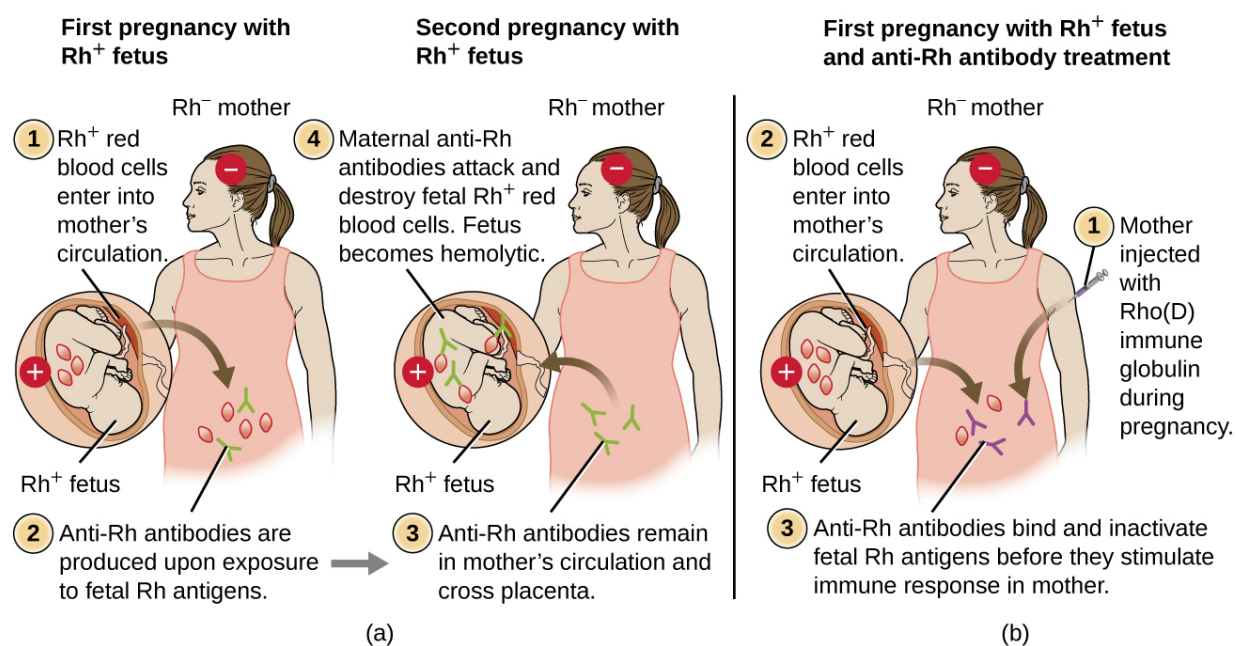
Rh factor incompatibility between mother and fetus can also cause a type II hypersensitivity hemolytic reaction, referred to as **hemolytic disease of the newborn (HDN)** ([\[link\]](#)). If an Rh⁻ woman carries an Rh⁺ baby to term, the mother's immune system can be exposed to Rh⁺ fetal red blood cells. This exposure will usually occur during the last trimester of pregnancy and during the delivery process. If this exposure occurs, the Rh⁺ fetal RBCs will activate a primary adaptive immune response in the mother, and anti-Rh factor IgG antibodies will be produced. IgG antibodies are the only class of antibody that can cross the placenta from mother to fetus; however, in most cases, the first Rh⁺ baby is unaffected by these antibodies because the first exposure typically occurs late enough in the pregnancy that the mother does not have time to mount a sufficient primary antibody response before the baby is born.

If a subsequent pregnancy with an Rh⁺ fetus occurs, however, the mother's second exposure to the Rh factor antigens causes a strong secondary antibody response that produces larger quantities of anti-Rh factor IgG. These antibodies can cross the placenta from mother to fetus and cause HDN, a potentially lethal condition for the baby ([\[link\]](#)).

Prior to the development of techniques for diagnosis and prevention, Rh factor incompatibility was the most common cause of HDN, resulting in thousands of infant deaths each year worldwide.[\[footnote\]](#) For this reason, the Rh factors of prospective parents are regularly screened, and treatments have been developed to prevent HDN caused by Rh incompatibility. To prevent Rh factor-mediated HDN, human Rho(D) immune globulin (e.g., RhoGAM) is injected intravenously or intramuscularly into the mother during the 28th week of pregnancy and within 72 hours after delivery. Additional doses may be administered after events that may result in

transplacental hemorrhage (e.g., umbilical blood sampling, chorionic villus sampling, abdominal trauma, amniocentesis). This treatment is initiated during the first pregnancy with an Rh⁺ fetus. The anti-Rh antibodies in Rho(D) immune globulin will bind to the Rh factor of any fetal RBCs that gain access to the mother's bloodstream, preventing these Rh⁺ cells from activating the mother's primary antibody response. Without a primary anti-Rh factor antibody response, the next pregnancy with an Rh⁺ will have minimal risk of HDN. However, the mother will need to be retreated with Rho(D) immune globulin during that pregnancy to prevent a primary anti-Rh antibody response that could threaten subsequent pregnancies.

G. Reali. "Forty Years of Anti-D Immunoprophylaxis." *Blood Transfusion* 5 no. 1 (2007):3–6.



(a) When an Rh⁻ mother has an Rh⁺ fetus, fetal erythrocytes are introduced into the mother's circulatory system before or during birth, leading to production of anti-Rh IgG antibodies. These antibodies remain in the mother and, if she becomes pregnant with a second Rh⁺ baby, they can cross the placenta and attach to fetal Rh⁺ erythrocytes. Complement-mediated hemolysis of fetal erythrocytes results in a lack of sufficient cells for proper oxygenation of the fetus. (b) HDN can be

prevented by administering Rho(D) immune globulin during and after each pregnancy with an Rh⁺ fetus. The immune globulin binds fetal Rh⁺ RBCs that gain access to the mother's bloodstream, preventing activation of her primary immune response.

Note:

Use this interactive [Blood Typing Game](#) to reinforce your knowledge of blood typing.

Note:

- What happens to cells that possess incompatible antigens in a type II hypersensitivity reaction?
- Describe hemolytic disease of the newborn and explain how it can be prevented.

Note:

Part 2

Kerry's primary care physician is not sure why Kerry seems to develop rashes after spending time in the sun, so she orders a urinalysis and basic blood tests. The results reveal that Kerry has proteinuria (abnormal protein levels in the urine), hemoglobinuria (excess hemoglobin in the urine), and a low hematocrit (RBC count). These tests suggest that Kerry is suffering from a mild bout of hemolytic anemia. The physician suspects that the problem might be autoimmune, so she refers Kerry to a rheumatologist for additional testing and diagnosis.

- Rheumatologists specialize in musculoskeletal diseases such as arthritis, osteoporosis, and joint pain. Why might Kerry's physician

refer her to this particular type of specialist even though she is exhibiting none of these symptoms?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Type III Hypersensitivities

Type III hypersensitivities are immune-complex reactions that were first characterized by Nicolas Maurice Arthus (1862–1945) in 1903. To produce antibodies for experimental procedures, Arthus immunized rabbits by injecting them with serum from horses. However, while immunizing rabbits repeatedly with horse serum, Arthus noticed a previously unreported and unexpected localized subcutaneous hemorrhage with edema at the site of injection. This reaction developed within 3 to 10 hours after injection. This localized reaction to non-self serum proteins was called an **Arthus reaction**. An Arthus reaction occurs when soluble antigens bind with IgG in a ratio that results in the accumulation of antigen-antibody aggregates called **immune complexes**.

A unique characteristic of **type III hypersensitivity** is antibody excess (primarily IgG), coupled with a relatively low concentration of antigen, resulting in the formation of small immune complexes that deposit on the surface of the epithelial cells lining the inner lumen of small blood vessels or on the surfaces of tissues ([\[link\]](#)). This immune complex accumulation leads to a cascade of inflammatory events that include the following:

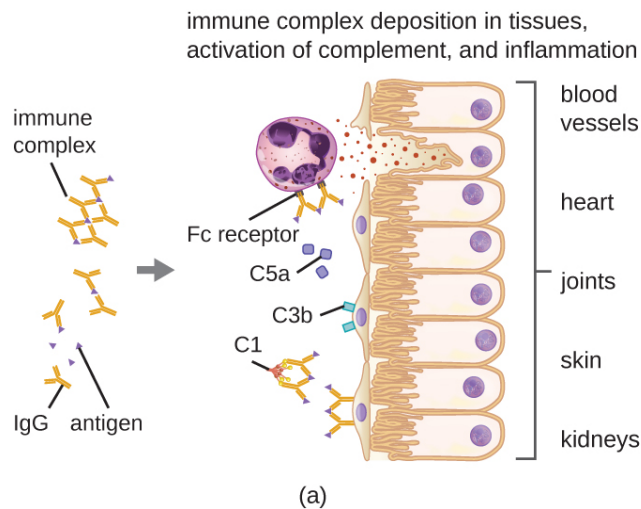
1. IgG binding to antibody receptors on localized mast cells, resulting in mast-cell degranulation
2. Complement activation with production of pro-inflammatory C3a and C5a (see [Chemical Defenses](#))
3. Increased blood-vessel permeability with chemotactic recruitment of neutrophils and macrophages

Because these immune complexes are not an optimal size and are deposited on cell surfaces, they cannot be phagocytosed in the usual way by

neutrophils and macrophages, which, in turn, are often described as “frustrated.” Although phagocytosis does not occur, neutrophil degranulation results in the release of lysosomal enzymes that cause extracellular destruction of the immune complex, damaging localized cells in the process. Activation of coagulation pathways also occurs, resulting in thrombi (blood clots) that occlude blood vessels and cause ischemia that can lead to vascular necrosis and localized hemorrhage.

Systemic type III hypersensitivity (**serum sickness**) occurs when immune complexes deposit in various body sites, resulting in a more generalized systemic inflammatory response. These immune complexes involve non-self proteins such as antibodies produced in animals for artificial passive immunity (see [Vaccines](#)), certain drugs, or microbial antigens that are continuously released over time during chronic infections (e.g., subacute bacterial endocarditis, chronic viral hepatitis). The mechanisms of serum sickness are similar to those described in localized type III hypersensitivity but involve widespread activation of mast cells, complement, neutrophils, and macrophages, which causes tissue destruction in areas such as the kidneys, joints, and blood vessels. As a result of tissue destruction, symptoms of serum sickness include chills, fever, rash, vasculitis, and arthritis. Development of glomerulonephritis or hepatitis is also possible.

Autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis can also involve damaging type III hypersensitivity reactions when auto-antibodies form immune complexes with self antigens. These conditions are discussed in [Autoimmune Disorders](#).



Type III hypersensitivities and the systems they affect. (a) Immune complexes form and deposit in tissue. Complement activation, stimulation of an inflammatory response, and recruitment and activation of neutrophils result in damage to blood vessels, heart tissue, joints, skin, and/or kidneys. (b) If the kidneys are damaged by a type III hypersensitivity reaction, dialysis may be required.

Note:

- Why is antibody excess important in type III hypersensitivity?
- Describe the differences between the Arthus reaction and serum sickness.

Note:

Diphtheria Antitoxin

Antibacterial sera are much less commonly used now than in the past, having been replaced by toxoid vaccines. However, a diphtheria antitoxin produced in horses is one example of such a treatment that is still used in some parts of the world. Although it is not licensed by the FDA for use in

the United States, diphtheria antitoxin can be used to treat cases of diphtheria, which are caused by the bacterium *Corynebacterium diphtheriae*.[\[footnote\]](#) The treatment is not without risks, however. Serum sickness can occur when the patient develops an immune response to non-self horse proteins. Immune complexes are formed between the horse proteins and circulating antibodies when the two exist in certain proportions. These immune complexes can deposit in organs, causing damage such as arthritis, nephritis, rash, and fever. Serum sickness is usually transient with no permanent damage unless the patient is chronically exposed to the antigen, which can then result in irreversible damage to body sites such as joints and kidneys. Over time, phagocytic cells such as macrophages are able to clear the horse serum antigens, which results in improvement of the patient's condition and a decrease in symptoms as the immune response dissipates.

Centers for Disease Control and Prevention. "Diphtheria Antitoxin." <http://www.cdc.gov/diphtheria/dat.html>. Accessed March 25, 2016.

Note:

Part 3

Kerry does not make it to the rheumatologist. She has a seizure as she is leaving her primary care physician's office. She is quickly rushed to the emergency department, where her primary care physician relates her medical history and recent test results. The emergency department physician calls in the rheumatologist on staff at the hospital for consultation. Based on the symptoms and test results, the rheumatologist suspects that Kerry has lupus and orders a pair of blood tests: an antinuclear antibody test (ANA) to look for antibodies that bind to DNA and another test that looks for antibodies that bind to a self-antigen called the Smith antigen (Sm).

- Based on the blood tests ordered, what type of reaction does the rheumatologist suspect is causing Kerry's seizure?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

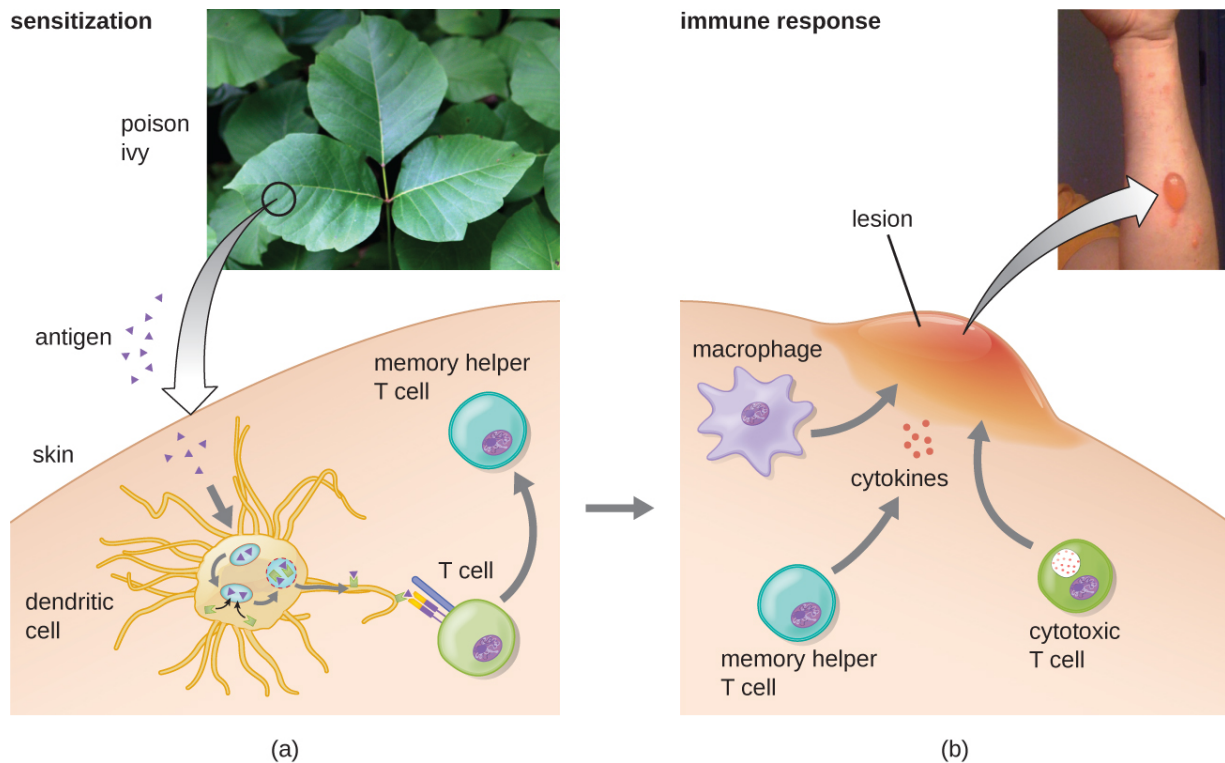
Type IV Hypersensitivities

Type IV hypersensitivities are not mediated by antibodies like the other three types of hypersensitivities. Rather, **type IV hypersensitivities** are regulated by T cells and involve the action of effector cells. These types of hypersensitivities can be organized into three subcategories based on T-cell subtype, type of antigen, and the resulting effector mechanism ([\[link\]](#)).

In the first type IV subcategory, CD4 T_H1-mediated reactions are described as delayed-type hypersensitivities (DTH). The sensitization step involves the introduction of antigen into the skin and phagocytosis by local antigen presenting cells (APCs). The APCs activate helper T cells, stimulating clonal proliferation and differentiation into memory T_H1 cells. Upon subsequent exposure to the antigen, these sensitized memory T_H1 cells release cytokines that activate macrophages, and activated macrophages are responsible for much of the tissue damage. Examples of this T_H1-mediated hypersensitivity are observed in tuberculin the Mantoux skin test and **contact dermatitis**, such as occurs in latex allergy reactions.

In the second type IV subcategory, CD4 T_H2-mediated reactions result in chronic asthma or chronic allergic rhinitis. In these cases, the soluble antigen is first inhaled, resulting in eosinophil recruitment and activation with the release of cytokines and inflammatory mediators.

In the third type IV subcategory, CD8 cytotoxic T lymphocyte (CTL)-mediated reactions are associated with tissue transplant rejection and contact dermatitis ([\[link\]](#)). For this form of cell-mediated hypersensitivity, APCs process and present the antigen with MHC I to naïve CD8 T cells. When these naïve CD8 T cells are activated, they proliferate and differentiate into CTLs. Activated T_H1 cells can also enhance the activation of the CTLs. The activated CTLs then target and induce granzyme-mediated apoptosis in cells presenting the same antigen with MHC I. These target cells could be “self” cells that have absorbed the foreign antigen (such as with contact dermatitis due to poison ivy), or they could be transplanted tissue cells displaying foreign antigen from the donor.



Exposure to hapten antigens in poison ivy can cause contact dermatitis, a type IV hypersensitivity. (a) The first exposure to poison ivy does not result in a reaction. However, sensitization stimulates helper T cells, leading to production of memory helper T cells that can become reactivated on future exposures. (b) Upon secondary exposure, the memory helper T cells become reactivated, producing inflammatory cytokines that stimulate macrophages and cytotoxic T cells to induce an inflammatory lesion at the exposed site. This lesion, which will persist until the allergen is removed, can inflict significant tissue damage if it continues long enough.

Type IV Hypersensitivities

Type IV Hypersensitivities Subcategory	Antigen	Effector Mechanism	Examples
Subcategory	Antigen	Effector Mechanism	Examples
1	Soluble antigen	Activated macrophages damage tissue and promote inflammatory response	Contact dermatitis (e.g., exposure to latex) and delayed-type hypersensitivity (e.g., tuberculin reaction)
2	Soluble antigen	Eosinophil recruitment and activation release cytokines and pro-inflammatory chemicals	Chronic asthma and chronic allergic rhinitis
3	Cell-associated antigen	CTL-mediated cytotoxicity	Contact dermatitis (e.g., contact with poison ivy) and tissue-transplant rejection

Note:

- Describe the three subtypes of type IV hypersensitivity.

- Explain how T cells contribute to tissue damage in type IV hypersensitivity.

Note:

Using Delayed Hypersensitivity to Test for TB

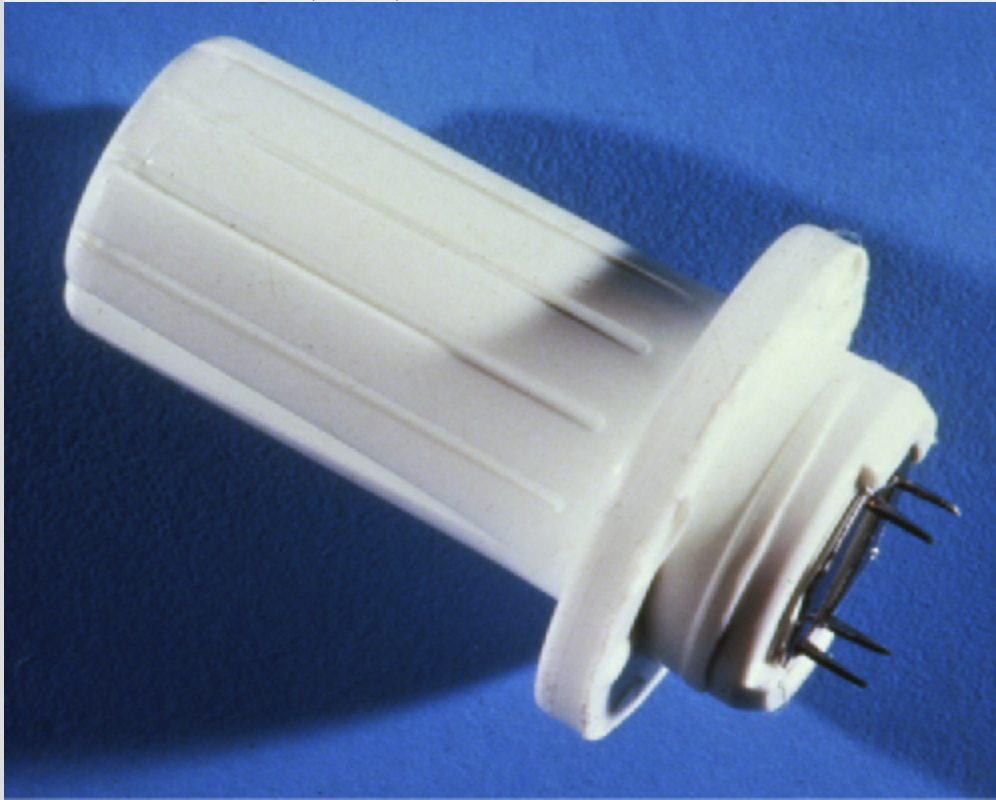
Austrian pediatrician Clemens von Pirquet (1874–1929) first described allergy mechanisms, including type III serum sickness.[\[footnote\]](#) His interest led to the development of a test for tuberculosis (TB), using the tuberculin antigen, based on earlier work identifying the TB pathogen performed by Robert Koch. Pirquet's method involved scarification, which results in simultaneous multiple punctures, using a device with an array of needles to break the skin numerous times in a small area. The device Pirquet used was similar to the tine test device with four needles seen in [\[link\]](#).

B. Huber "100 Jahre Allergie: Clemens von Pirquet—sein Allergiebegriff und das ihm zugrunde liegende Krankheitsverständnis." *Wiener Klinische Wochenschrift* 118 no. 19–20 (2006):573–579.

The tips of all the needles in the array are coated with tuberculin, a protein extract of TB bacteria, effectively introducing the tuberculin into the skin. One to 3 days later, the area can be examined for a delayed hypersensitivity reaction, signs of which include swelling and redness.

As you can imagine, scarification was not a pleasant experience,[\[footnote\]](#) and the numerous skin punctures put the patient at risk of developing bacterial infection of the skin. Mantoux modified Pirquet's test to use a single subcutaneous injection of purified tuberculin material. A positive test, which is indicated by a delayed localized swelling at the injection site, does not necessarily mean that the patient is currently infected with active TB. Because type IV (delayed-type) hypersensitivity is mediated by reactivation of memory T cells, such cells may have been created recently (due to an active current infection) or years prior (if a patient had TB and had spontaneously cleared it, or if it had gone into latency). However, the test can be used to confirm infection in cases in which symptoms in the patient or findings on a radiograph suggest its presence.

C.A. Stewart. "The Pirquet Test: Comparison of the Scarification and the Puncture Methods of Application." *Archives of Pediatrics & Adolescent Medicine* 35 no. 3 (1928):388–391.



The modern version of Pirquet's scarification is the tine test, which uses devices like this to administer tuberculin antigen into the skin, usually on the inside of the forearm. The tine test is considered less reliable than the Mantoux test. (credit: modification of work by the Centers for Disease Control and Prevention)

Hypersensitivity Pneumonitis

Some disease caused by hypersensitivities are not caused exclusively by one type. For example, **hypersensitivity pneumonitis (HP)**, which is often an occupational or environmental disease, occurs when the lungs become

inflamed due to an allergic reaction to inhaled dust, endospores, bird feathers, bird droppings, molds, or chemicals. HP goes by many different names associated with various forms of exposure ([link](#)). HP associated with bird droppings is sometimes called pigeon fancier's lung or poultry worker's lung—both common in bird breeders and handlers. Cheese handler's disease, farmer's lung, sauna takers' disease, and hot-tub lung are other names for HP associated with exposure to molds in various environments.

Pathology associated with HP can be due to both type III (mediated by immune complexes) and type IV (mediated by T_H1 cells and macrophages) hypersensitivities. Repeated exposure to allergens can cause alveolitis due to the formation of immune complexes in the alveolar wall of the lung accompanied by fluid accumulation, and the formation of granulomas and other lesions in the lung as a result of T_H1 -mediated macrophage activation. Alveolitis with fluid and granuloma formation results in poor oxygen perfusion in the alveoli, which, in turn, can cause symptoms such as coughing, dyspnea, chills, fever, sweating, myalgias, headache, and nausea. Symptoms may occur as quickly as 2 hours after exposure and can persist for weeks if left untreated.



(a)



(b)

Occupational exposure to dust, mold, and other allergens can result in hypersensitivity pneumonitis. (a) People exposed daily to large numbers of birds may be susceptible to poultry worker's lung. (b)

Workers in a cheese factory may become sensitized to different types of molds and develop cheese handler's disease. (credit a: modification of work by The Global Orphan Project)

Note:

- Explain why hypersensitivity pneumonitis is considered an occupational disease.

[\[link\]](#) summarizes the mechanisms and effects of each type of hypersensitivity discussed in this section.

Hypersensitivity Types and Their Mechanisms				
	Type I	Type II	Type III	Type IV
Immune reactant	IgE	IgG or IgM	IgG and IgM	T cells
Antigen form	Soluble antigen	Cell-bound antigen	Soluble antigen	Soluble or cell-bound antigen
Mechanism of activation	Allergen-specific IgE antibodies bind to mast cells via their Fc receptor. When the specific allergen binds to the IgE, cross-linking of IgE induces degranulation of mast cells.	IgG or IgM antibody binds to cellular antigen, leading to complement activation and cell lysis. IgG can also mediate ADCC with cytotoxic T cells, natural killer cells, macrophages, and neutrophils.	Antigen-antibody complexes are deposited in tissues. Complement activation provides inflammatory mediators and recruits neutrophils. Enzymes released from neutrophils damage tissue.	T _H 1 cells secrete cytokines, which activate macrophages and cytotoxic T cells.
Examples of hypersensitivity reactions	Local and systemic anaphylaxis, seasonal hay fever, food allergies, and drug allergies	Red blood cell destruction after transfusion with mismatched blood types or during hemolytic disease of the newborn.	Post-streptococcal glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus	Contact dermatitis, type I diabetes mellitus, and multiple sclerosis

Components of the immune system cause four types of hypersensitivities. Notice that types I–III are B-cell/antibody-mediated hypersensitivities, whereas type IV hypersensitivity is exclusively a T-cell phenomenon.

Diagnosis of Hypersensitivities

Diagnosis of type I hypersensitivities is a complex process requiring several diagnostic tests in addition to a well-documented patient history. Serum IgE levels can be measured, but elevated IgE alone does not confirm allergic disease. As part of the process to identify the antigens responsible for a type I reaction allergy, testing through a prick puncture skin test (PPST) or an intradermal test can be performed. PPST is carried out with the introduction of allergens in a series of superficial skin pricks on the patient's back or arms ([\[link\]](#)). PPSTs are considered to be the most convenient and least

expensive way to diagnose allergies, according to the US Joint Council of Allergy and the European Academy of Allergy and Immunology. The second type of testing, the intradermal test, requires injection into the dermis with a small needle. This needle, also known as a tuberculin needle, is attached to a syringe containing a small amount of allergen. Both the PPST and the intradermal tests are observed for 15–20 minutes for a **wheal-flare reaction** to the allergens. Measurement of any wheal (a raised, itchy bump) and flare (redness) within minutes indicates a type I hypersensitivity, and the larger the wheal-flare reaction, the greater the patient's sensitivity to the allergen.

Type III hypersensitivities can often be misdiagnosed because of their nonspecific inflammatory nature. The symptoms are easily visible, but they may be associated with any of a number of other diseases. A strong, comprehensive patient history is crucial to proper and accurate diagnosis. Tests used to establish the diagnosis of hypersensitivity pneumonitis (resulting from type III hypersensitivity) include bronchoalveolar lavage (BAL), pulmonary function tests, and high-resolution computed tomography (HRCT).



Results of an allergy skin-prick test to test for type I hypersensitivity to a group of potential allergens. A positive result is indicated by a raised area (wheal) and surrounding redness (flare). (credit: modification of work by “OakleyOriginals”/Flickr)

Note:

- Describe the prick puncture skin test.
- Explain why type III hypersensitivities can be difficult to diagnose.

Treatments of Hypersensitivities

Allergic reactions can be treated in various ways. Prevention of allergic reactions can be achieved by **desensitization** (hyposensitization) therapy, which can be used to reduce the hypersensitivity reaction through repeated injections of allergens. Extremely dilute concentrations of known allergens (determined from the allergen tests) are injected into the patient at prescribed intervals (e.g., weekly). The quantity of allergen delivered by the shots is slowly increased over a buildup period until an effective dose is determined and that dose is maintained for the duration of treatment, which can last years. Patients are usually encouraged to remain in the doctor's office for 30 minutes after receiving the injection in case the allergens administered cause a severe systemic reaction. Doctors' offices that administer desensitization therapy must be prepared to provide resuscitation and drug treatment in the case of such an event.

Desensitization therapy is used for insect sting allergies and environmental allergies. The allergy shots elicit the production of different interleukins and IgG antibody responses instead of IgE. When excess allergen-specific IgG antibodies are produced and bind to the allergen, they can act as **blocking antibodies** to neutralize the allergen before it can bind IgE on mast cells. There are early studies using oral therapy for desensitization of food allergies that are promising.[\[footnote\]](#)[\[footnote\]](#) These studies involve feeding children who have allergies tiny amounts of the allergen (e.g., peanut flour) or related proteins over time. Many of the subjects show reduced severity of reaction to the food allergen after the therapy.

C.L. Schneider et al. "A Pilot Study of Omalizumab to Facilitate Rapid Oral Desensitization in High-Risk Peanut-Allergic Patients." *Journal of Allergy and Clinical Immunology* 132 no. 6 (2013):1368–1374.

P. Varshney et al. "A Randomized Controlled Study of Peanut Oral Immunotherapy: Clinical Desensitization and Modulation of the Allergic

Response.” *Journal of Allergy and Clinical Immunology* 127 no. 3 (2011):654–660.

There are also therapies designed to treat severe allergic reactions. Emergency systemic anaphylaxis is treated initially with an epinephrine injection, which can counteract the drop in blood pressure. Individuals with known severe allergies often carry a self-administering auto-injector that can be used in case of exposure to the allergen (e.g., an insect sting or accidental ingestion of a food that causes a severe reaction). By self-administering an epinephrine shot (or sometimes two), the patient can stem the reaction long enough to seek medical attention. Follow-up treatment generally involves giving the patient antihistamines and slow-acting corticosteroids for several days after the reaction to prevent potential late-phase reactions. However, the effects of antihistamine and corticosteroid treatment are not well studied and are used based on theoretical considerations.

Treatment of milder allergic reactions typically involves antihistamines and other anti-inflammatory drugs. A variety of antihistamine drugs are available, in both prescription and over-the-counter strengths. There are also antileukotriene and antiprostaglandin drugs that can be used in tandem with antihistamine drugs in a combined (and more effective) therapy regime.

Treatments of type III hypersensitivities include preventing further exposure to the antigen and the use of anti-inflammatory drugs. Some conditions can be resolved when exposure to the antigen is prevented. Anti-inflammatory corticosteroid inhalers can also be used to diminish inflammation to allow lung lesions to heal. Systemic corticosteroid treatment, oral or intravenous, is also common for type III hypersensitivities affecting body systems. Treatment of hypersensitivity pneumonitis includes avoiding the allergen, along with the possible addition of prescription steroids such as prednisone to reduce inflammation.

Treatment of type IV hypersensitivities includes antihistamines, anti-inflammatory drugs, analgesics, and, if possible, eliminating further exposure to the antigen.

Note:

- Describe desensitization therapy.
- Explain the role of epinephrine in treatment of hypersensitivity reactions.

Key Concepts and Summary

- An **allergy** is an adaptive immune response, sometimes life-threatening, to an **allergen**.
- **Type I hypersensitivity** requires sensitization of mast cells with IgE, involving an initial IgE antibody response and IgE attachment to mast cells. On second exposure to an allergen, cross-linking of IgE molecules on mast cells triggers degranulation and release of preformed and newly formed chemical mediators of inflammation. Type I hypersensitivity may be localized and relatively minor (hives and hay fever) or system-wide and dangerous (systemic **anaphylaxis**).
- **Type II hypersensitivities** result from antibodies binding to antigens on cells and initiating cytotoxic responses. Examples include **hemolytic transfusion reaction** and **hemolytic disease of the newborn**.
- **Type III hypersensitivities** result from formation and accumulation of **immune complexes** in tissues, stimulating damaging inflammatory responses.
- **Type IV hypersensitivities** are not mediated by antibodies, but by helper T-cell activation of macrophages, eosinophils, and cytotoxic T cells.

Multiple Choice**Exercise:**

Problem:

Which of the following is the type of cell largely responsible for type I hypersensitivity responses?

- A. erythrocyte
- B. mast cell
- C. T lymphocyte
- D. antibody

Solution:

B

Exercise:**Problem:**

Type I hypersensitivities require which of the following initial priming events to occur?

- A. sensitization
- B. secondary immune response
- C. cellular trauma
- D. degranulation

Solution:

A

Exercise:**Problem:**

Which of the following are the main mediators/initiators of type II hypersensitivity reactions?

- A. antibodies

- B. mast cells
- C. erythrocytes
- D. histamines

Solution:

A

Exercise:

Problem:

Inflammatory molecules are released by mast cells in type I hypersensitivities; type II hypersensitivities, however, are characterized by which of the following?

- A. cell lysis (cytotoxicity)
- B. strong antibody reactions against antigens
- C. leukotriene release upon stimulation
- D. localized tissue reactions, such as hives

Solution:

A

Exercise:

Problem:

An immune complex is an aggregate of which of the following?

- A. antibody molecules
- B. antigen molecules
- C. antibody and antigen molecules
- D. histamine molecules

Solution:

C

Exercise:

Problem:

Which of the following is a common treatment for type III hypersensitivity reactions?

- A. anti-inflammatory steroid treatments
- B. antihistamine treatments
- C. hyposensitization injections of allergens
- D. RhoGAM injections

Solution:

A

Exercise:

Problem: Which of the following induces a type III hypersensitivity?

- A. release of inflammatory molecules from mast cells
- B. accumulation of immune complexes in tissues and small blood vessels
- C. destruction of cells bound by antigens
- D. destruction of cells bound by antibodies

Solution:

B

Exercise:

Problem:

Which one of the following is not an example of a type IV hypersensitivity?

- A. latex allergy
 - B. Contact dermatitis (e.g., contact with poison ivy)
 - C. a positive tuberculin skin test
 - D. hemolytic disease of the newborn
-

Solution:

D

Fill in the Blank

Exercise:

Problem:

Antibodies involved in type I hypersensitivities are of the _____ class.

Solution:

IgE

Exercise:

Problem:

Allergy shots work by shifting antibody responses to produce _____ antibodies.

Solution:

IgG

Exercise:

Problem:

A person who is blood type A would have IgM hemagglutinin antibodies against type _____ red blood cells in their plasma.

Solution:

B

Exercise:**Problem:**

The itchy and blistering rash that develops with contact to poison ivy is caused by a type _____ hypersensitivity reaction.

Solution:

IV

Short Answer**Exercise:****Problem:**

Although both type I and type II hypersensitivities involve antibodies as immune effectors, different mechanisms are involved with these different hypersensitivities. Differentiate the two.

Exercise:**Problem:**

What types of antibodies are most common in type III hypersensitivities, and why?

Critical Thinking**Exercise:**

Problem:

Patients are frequently given instructions to avoid allergy medications for a period of time prior to allergy testing. Why would this be important?

Exercise:**Problem:**

In some areas of the world, a tuberculosis vaccine known as bacillus Calmette-Guérin (BCG) is used. It is not used in the United States. Every person who has received this vaccine and mounted a protective response will have a positive reaction in a tuberculin skin test. Why? What does this mean for the usefulness of this skin test in those countries where this vaccine is used?

Autoimmune Disorders

LEARNING OBJECTIVES

- Explain why autoimmune disorders develop
- Provide a few examples of organ-specific and systemic autoimmune diseases

In 1970, artist Walt Kelly developed a poster promoting Earth Day, featuring a character from *Pogo*, his daily newspaper comic strip. In the poster, Pogo looks out across a litter-strewn forest and says wryly, “We have met the enemy and he is us.” Pogo was not talking about the human immune system, but he very well could have been. Although the immune system protects the body by attacking invading “enemies” (pathogens), in some cases, the immune system can mistakenly identify the body’s own cells as the enemy, resulting in **autoimmune disease**.

Autoimmune diseases are those in which the body is attacked by its own specific adaptive immune response. In normal, healthy states, the immune system induces **tolerance**, which is a lack of an anti-self immune response. However, with autoimmunity, there is a loss of immune tolerance, and the mechanisms responsible for autoimmune diseases include type II, III, and IV hypersensitivity reactions. Autoimmune diseases can have a variety of mixed symptoms that flare up and disappear, making diagnosis difficult.

The causes of autoimmune disease are a combination of the individual's genetic makeup and the effect of environmental influences, such as sunlight, infections, medications, and environmental chemicals. However, the vagueness of this list reflects our poor understanding of the etiology of

these diseases. Except in a very few specific diseases, the initiation event(s) of most autoimmune states has not been fully characterized.

There are several possible causes for the origin of autoimmune diseases and autoimmunity is likely due to several factors. Evidence now suggests that regulatory T and B cells play an essential role in the maintenance of tolerance and prevention of autoimmune responses. The regulatory T cells are especially important for inhibiting autoreactive T cells that are not eliminated during thymic selection and escape the thymus (see [T Lymphocytes and Cellular Immunity](#)). In addition, antigen mimicry between pathogen antigens and our own self antigens can lead to cross-reactivity and autoimmunity. Hidden self-antigens may become exposed because of trauma, drug interactions, or disease states, and trigger an autoimmune response. All of these factors could contribute to autoimmunity. Ultimately, damage to tissues and organs in the autoimmune disease state comes as a result of inflammatory responses that are inappropriate; therefore, treatment often includes immunosuppressive drugs and corticosteroids.

Organ-Specific Autoimmune Diseases

Some autoimmune diseases are considered organ specific, meaning that the immune system targets specific organs or tissues. Examples of organ-specific autoimmune diseases include celiac disease, Graves disease, Hashimoto thyroiditis, type I diabetes mellitus, and Addison disease.

Celiac Disease

Celiac disease is largely a disease of the small intestine, although other organs may be affected. People in their 30s and 40s, and children are most commonly affected, but **celiac disease** can begin at any age. It results from a reaction to proteins, commonly called gluten, found mainly in wheat, barley, rye, and some other grains. The disease has several genetic causes (predispositions) and poorly understood environmental influences. On exposure to gluten, the body produces various autoantibodies and an

inflammatory response. The inflammatory response in the small intestine leads to a reduction in the depth of the microvilli of the mucosa, which hinders absorption and can lead to weight loss and anemia. The disease is also characterized by diarrhea and abdominal pain, symptoms that are often misdiagnosed as irritable bowel syndrome.

Diagnosis of celiac disease is accomplished from serological tests for the presence of primarily IgA antibodies to components of gluten, the transglutaminase enzyme, and autoantibodies to endomysium, a connective tissue surrounding muscle fibers. Serological tests are typically followed up with endoscopy and biopsy of the duodenal mucosa. Serological screening surveys have found about 1% of individuals in the United Kingdom are positive even though they do not all display symptoms.[\[footnote\]](#) This early recognition allows for more careful monitoring and prevention of severe disease.

D.A. Van Heel, J. West. “Recent Advances in Coeliac Disease.” *Gut* 55 no. 7 (2006):1037—1046.

Celiac disease is treated with complete removal of gluten-containing foods from the diet, which results in improved symptoms and reduced risk of complications. Other theoretical approaches include breeding grains that do not contain the immunologically reactive components or developing dietary supplements that contain enzymes that break down the protein components that cause the immune response.[\[footnote\]](#)
ibid.

Disorders of the Thyroid

Graves disease is the most common cause of hyperthyroidism in the United States. Symptoms of Graves disease result from the production of thyroid-stimulating immunoglobulin (TSI) also called TSH-receptor antibody. TSI targets and binds to the receptor for thyroid stimulating hormone (TSH), which is naturally produced by the pituitary gland. TSI may cause conflicting symptoms because it may stimulate the thyroid to make too much thyroid hormone or block thyroid hormone production entirely, making diagnosis more difficult. Signs and symptoms of Graves disease

include heat intolerance, rapid and irregular heartbeat, weight loss, goiter (a swollen thyroid gland, protruding under the skin of the throat [\[link\]](#)) and exophthalmia (bulging eyes) often referred to as Graves ophthalmopathy ([link](#)).

The most common cause of hypothyroidism in the United States is **Hashimoto thyroiditis**, also called chronic lymphocytic thyroiditis. Patients with Hashimoto thyroiditis often develop a spectrum of different diseases because they are more likely to develop additional autoimmune diseases such as Addison disease (discussed later in this section), type 1 diabetes, rheumatoid arthritis, and celiac disease. Hashimoto thyroiditis is a T_H1 cell-mediated disease that occurs when the thyroid gland is attacked by cytotoxic lymphocytes, macrophages, and autoantibodies. This autoimmune response leads to numerous symptoms that include goiter ([link](#)), cold intolerance, muscle weakness, painful and stiff joints, depression, and memory loss.



Goiter, a hypertrophy of the thyroid, is a symptom of

Graves disease and Hashimoto thyroiditis.



Exophthalmia, or Graves ophthalmopathy, is a sign of Graves disease.
(credit: modification of work by Jonathan Trobe, University of
Michigan Kellogg Eye Center)

Type 1 Diabetes

Juvenile diabetes, or **type 1 diabetes mellitus**, is usually diagnosed in children and young adults. It is a T-cell-dependent autoimmune disease characterized by the selective destruction of the β cells of the islets of

Langerhans in the pancreas by CD4 T_H1-mediated CD8 T cells, anti-β-cell antibodies, and macrophage activity. There is also evidence that viral infections can have either a potentiating or inhibitory role in the development of type 1 diabetes (T1D) mellitus. The destruction of the β cells causes a lack of insulin production by the pancreas. In T1D, β-cell destruction may take place over several years, but symptoms of hyperglycemia, extreme increase in thirst and urination, weight loss, and extreme fatigue usually have a sudden onset, and diagnosis usually does not occur until most β cells have already been destroyed.

Autoimmune Addison Disease

Destruction of the adrenal glands (the glands lying above the kidneys that produce glucocorticoids, mineralocorticoids, and sex steroids) is the cause of **Addison disease**, also called primary adrenal insufficiency (PAI). Today, up to 80% of Addison disease cases are diagnosed as autoimmune Addison disease (AAD), which is caused by an autoimmune response to adrenal tissues disrupting adrenal function. Disruption of adrenal function causes impaired metabolic processes that require normal steroid hormone levels, causing signs and symptoms throughout the body. There is evidence that both humoral and CD4 T_H1-driven CD8 T-cell-mediated immune mechanisms are directed at the adrenal cortex in AAD. There is also evidence that the autoimmune response is associated with autoimmune destruction of other endocrine glands as well, such as the pancreas and thyroid, conditions collectively referred to as autoimmune polyendocrine syndromes (APS). In up to 80% of patients with AAD, antibodies are produced to three enzymes involved in steroid synthesis: 21-hydroxylase (21-OH), 17α-hydroxylase, and cholesterol side-chain-cleaving enzyme. [\[footnote\]](#) The most common autoantibody found in AAD is to 21-OH, and antibodies to any of the key enzymes for steroid production are diagnostic for AAD. The adrenal cortex cells are targeted, destroyed, and replaced with fibrous tissue by immune-mediated inflammation. In some patients, at least 90% of the adrenal cortex is destroyed before symptoms become diagnostic.

P. Martorell et al. “Autoimmunity in Addison’s Disease.” *Netherlands Journal of Medicine* 60 no. 7 (2002):269—275.

Symptoms of AAD include weakness, nausea, decreased appetite, weight loss, hyperpigmentation ([\[link\]](#)), hyperkalemia (elevated blood potassium levels), hyponatremia (decreased blood sodium levels), hypoglycemia (decreased levels of blood sugar), hypotension (decreased blood pressure), anemia, lymphocytosis (decreased levels of white blood cells), and fatigue. Under extreme stress, such as surgery, accidental trauma, or infection, patients with AAD may experience an adrenal crisis that causes the patient to vomit, experience abdominal pain, back or leg cramps, and even severe hypotension leading to shock.



Hyperpigmentation is a sign of Addison disease. (credit: modification of work by Petros Perros)

Note:

- What are the names of autoimmune diseases that interfere with hormone gland function?
- Describe how the mechanisms of Graves disease and Hashimoto thyroiditis differ.
- Name the cells that are destroyed in type 1 diabetes mellitus and describe the result.

Systemic Autoimmune Diseases

Whereas organ-specific autoimmune diseases target specific organs or tissues, **systemic autoimmune diseases** are more generalized, targeting multiple organs or tissues throughout the body. Examples of systemic autoimmune diseases include multiple sclerosis, myasthenia gravis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus.

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune central nervous system disease that affects the brain and spinal cord. Lesions in multiple locations within the central nervous system are a hallmark of **multiple sclerosis** and are caused by infiltration of immune cells across the blood-brain barrier. The immune cells include T cells that promote inflammation, demyelination, and neuron degeneration, all of which disrupt neuronal signaling.

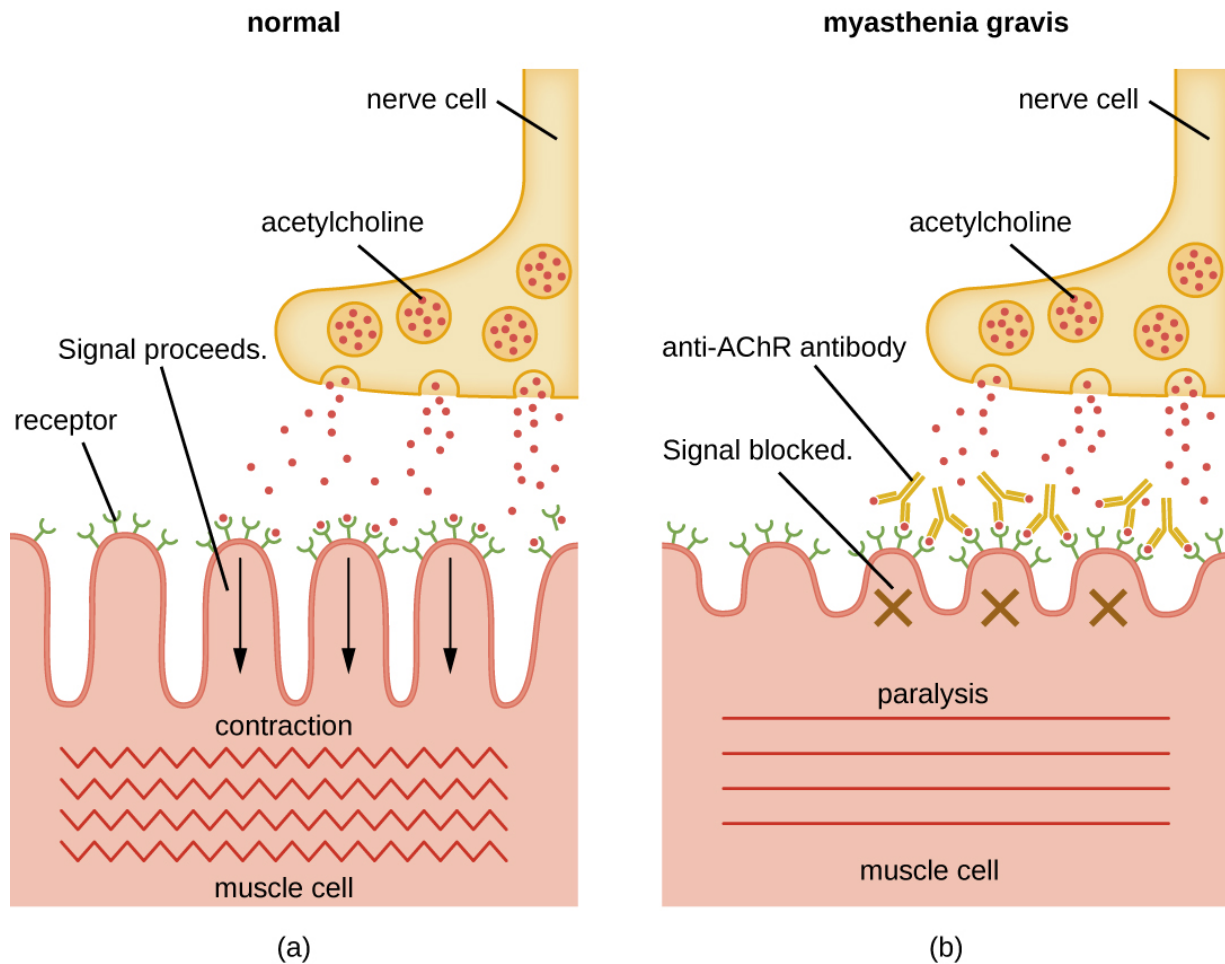
Symptoms of MS include visual disturbances; muscle weakness; difficulty with coordination and balance; sensations such as numbness, prickling, or “pins and needles”; and cognitive and memory problems.

Myasthenia Gravis

Autoantibodies directed against acetylcholine receptors (AChRs) in the synaptic cleft of neuromuscular junctions lead to **myasthenia gravis** ([link](#)). Anti-AChR antibodies are high-affinity IgGs and their synthesis requires activated CD4 T cells to interact with and stimulate B cells. Once produced, the anti-AChR antibodies affect neuromuscular transmission by at least three mechanisms:

- Complement binding and activation at the neuromuscular junction
- Accelerated AChR endocytosis of molecules cross-linked by antibodies
- Functional AChR blocking, which prevents normal acetylcholine attachment to, and activation of, AChR

Regardless of the mechanism, the effect of anti-AChR is extreme muscle weakness and potentially death through respiratory arrest in severe cases.



Myasthenia gravis and impaired muscle contraction. (a) Normal release of the neurotransmitter acetylcholine stimulates muscle contraction. (b) In myasthenia gravis, autoantibodies block the receptors for acetylcholine (AChR) on muscle cells, resulting in paralysis.

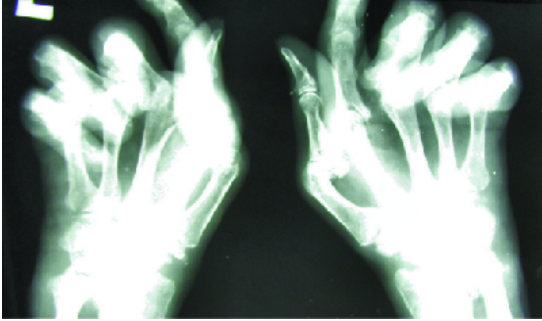
Psoriasis

Psoriasis is a skin disease that causes itchy or sore patches of thick, red skin with silvery scales on elbows, knees, scalp, back, face, palms, feet, and sometimes other areas. Some individuals with **psoriasis** also get a form of arthritis called psoriatic arthritis, in which the joints can become inflamed.

Psoriasis results from the complex interplay between keratinocytes, dendritic cells, and T cells, and the cytokines produced by these various cells. In a process called cell turnover, skin cells that grow deep in the skin rise to the surface. Normally, this process takes a month. In psoriasis, as a result of cytokine activation, cell turnover happens in just a few days. The thick inflamed patches of skin that are characteristic of psoriasis develop because the skin cells rise too fast.

Rheumatoid Arthritis

The most common chronic inflammatory joint disease is **rheumatoid arthritis** (RA) ([\[link\]](#)) and it is still a major medical challenge because of unsolved questions related to the environmental and genetic causes of the disease. RA involves type III hypersensitivity reactions and the activation of CD4 T cells, resulting in chronic release of the inflammatory cytokines IL-1, IL-6, and tumor necrosis factor- α (TNF- α). The activated CD4 T cells also stimulate the production of rheumatoid factor (RF) antibodies and anticyclic citrullinated peptide antibodies (anti-CCP) that form immune complexes. Increased levels of acute-phase proteins, such as C-reactive protein (CRP), are also produced as part of the inflammatory process and participate in complement fixation with the antibodies on the immune complexes. The formation of immune complexes and reaction to the immune factors cause an inflammatory process in joints, particularly in the hands, feet, and legs. Diagnosis of RA is based on elevated levels of RF, anti-CCP, quantitative CRP, and the erythrocyte sedimentation rate (ESR) (modified Westergren). In addition, radiographs, ultrasound, or magnetic resonance imaging scans can identify joint damage, such as erosions, a loss of bone within the joint, and narrowing of joint space.



The radiograph (left) and photograph (right) show damage to the hands typical of rheumatoid arthritis. (credit right: modification of work by “handarmdoc”/Flickr)

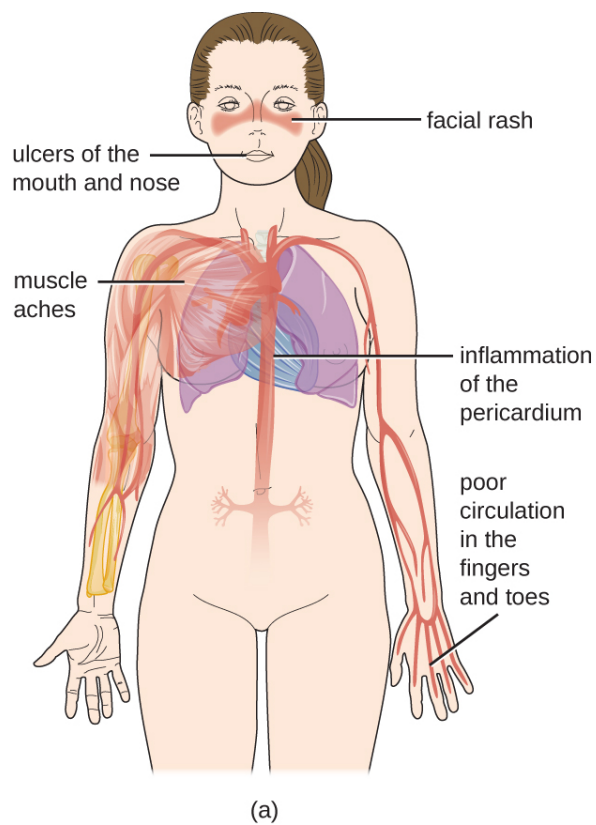
Systemic Lupus Erythematosus

The damage and pathology of **systemic lupus erythematosus (SLE)** is caused by type III hypersensitivity reactions. Autoantibodies produced in SLE are directed against nuclear and cytoplasmic proteins. Anti-nuclear antibodies (ANAs) are present in more than 95% of patients with SLE, [\[footnote\]](#) with additional autoantibodies including anti-double-stranded DNA (ds-DNA) and anti-Sm antibodies (antibodies to small nuclear ribonucleoprotein). Anti-ds-DNA and anti-Sm antibodies are unique to patients with SLE; thus, their presence is included in the classification criteria of SLE. Cellular interaction with autoantibodies leads to nuclear and cellular destruction, with components released after cell death leading to the formation of immune complexes.

C.C. Mok, C.S. Lau. “Pathogenesis of Systemic Lupus Erythematosus.” *Journal of Clinical Pathology* 56 no. 7 (2003):481—490.

Because autoantibodies in SLE can target a wide variety of cells, symptoms of SLE can occur in many body locations. However, the most common symptoms include fatigue, fever with no other cause, hair loss, and a sunlight-sensitive "butterfly" or wolf-mask (lupus) rash that is found in about 50% of people with SLE ([\[link\]](#)). The rash is most often seen over the cheeks and bridge of the nose, but can be widespread. Other symptoms may

appear depending on affected areas. The joints may be affected, leading to arthritis of the fingers, hands, wrists, and knees. Effects on the brain and nervous system can lead to headaches, numbness, tingling, seizures, vision problems, and personality changes. There may also be abdominal pain, nausea, vomiting, arrhythmias, shortness of breath, and blood in the sputum. Effects on the skin can lead to additional areas of skin lesions, and vasoconstriction can cause color changes in the fingers when they are cold (Raynaud phenomenon). Effects on the kidneys can lead to edema in the legs and weight gain. A diagnosis of SLE depends on identification of four of 11 of the most common symptoms and confirmed production of an array of autoantibodies unique to SLE. A positive test for ANAs alone is not diagnostic.



(a) Systemic lupus erythematosus is characterized by autoimmunity to the individual's own DNA and/or proteins. (b) This patient is presenting with a butterfly rash, one of the characteristic signs of lupus. (credit a: modification of work by Mikael Häggström; credit b:

modification of work by Shrestha D, Dhakal AK, Shiva RK, Shakya A, Shah SC, Shakya H)

Note:

- List the ways antibodies contribute to the pathogenesis of myasthenia gravis.
- Explain why rheumatoid arthritis is considered a type III hypersensitivity.
- Describe the symptoms of systemic lupus erythematosus and explain why they affect so many different parts of the body.
- What is recognized as an antigen in myasthenia gravis?

[\[link\]](#) summarizes the causes, signs, and symptoms of select autoimmune diseases.

Select Autoimmune Diseases		
Disease	Cause	Signs and Symptoms

Select Autoimmune Diseases		
Disease	Cause	Signs and Symptoms
Addison disease	Destruction of adrenal gland cells by cytotoxic T cells	Weakness, nausea, hypotension, fatigue; adrenal crisis with severe pain in abdomen, lower back, and legs; circulatory system collapse, kidney failure
Celiac disease	Antibodies to gluten become autoantibodies that target cells of the small intestine	Severe diarrhea, abdominal pain, anemia, malnutrition
Diabetes mellitus (type I)	Cytotoxic T-cell destruction of the insulin-producing β cells of the pancreas	Hyperglycemia, extreme increase in thirst and urination, weight loss, extreme fatigue
Graves disease	Autoantibodies target thyroid-stimulating hormone receptors, resulting in overstimulation of the thyroid	Hyperthyroidism with rapid and irregular heartbeat, heat intolerance, weight loss, goiter, exophthalmia

Select Autoimmune Diseases		
Disease	Cause	Signs and Symptoms
Hashimoto thyroiditis	Thyroid gland is attacked by cytotoxic T cells, lymphocytes, macrophages, and autoantibodies	Thyroiditis with goiter, cold intolerance, muscle weakness, painful and stiff joints, depression, memory loss
Multiple sclerosis (MS)	Cytotoxic T-cell destruction of the myelin sheath surrounding nerve axons in the central nervous system	Visual disturbances, muscle weakness, impaired coordination and balance, numbness, prickling or “pins and needles” sensations, impaired cognitive function and memory
Myasthenia gravis	Autoantibodies directed against acetylcholine receptors within the neuromuscular junction	Extreme muscle weakness eventually leading to fatal respiratory arrest
Psoriasis	Cytokine activation of keratinocytes causes rapid and excessive epidermal cell turnover	Itchy or sore patches of thick, red skin with silvery scales; commonly affects elbows, knees, scalp, back, face, palms, feet

Select Autoimmune Diseases		
Disease	Cause	Signs and Symptoms
Rheumatoid arthritis	Autoantibodies, immune complexes, complement activation, phagocytes, and T cells damage membranes and bone in joints	Joint inflammation, pain and disfigurement, chronic systemic inflammation
Systemic lupus erythematosus (SLE)	Autoantibodies directed against nuclear and cytoplasmic molecules form immune complexes that deposit in tissues. Phagocytic cells and complement activation cause tissue damage and inflammation	Fatigue, fever, joint pain and swelling, hair loss, anemia, clotting, a sunlight-sensitive "butterfly" rash, skin lesions, photosensitivity, decreased kidney function, memory loss, confusion, depression

Summary

- **Autoimmune diseases** result from a breakdown in immunological tolerance. The actual induction event(s) for autoimmune states are largely unknown.
- Some autoimmune diseases attack specific organs, whereas others are more systemic.
- Organ-specific autoimmune diseases include **celiac disease, Graves disease, Hashimoto thyroiditis, type I diabetes mellitus, and Addison disease.**

- Systemic autoimmune diseases include **multiple sclerosis**, **myasthenia gravis**, **psoriasis**, **rheumatoid arthritis**, and **systemic lupus erythematosus**.
- Treatments for autoimmune diseases generally involve anti-inflammatory and immunosuppressive drugs.

Multiple Choice

Exercise:

Problem:

Which of the following is an example of an organ-specific autoimmune disease?

- A. rheumatoid arthritis
- B. psoriasis
- C. Addison disease
- D. myasthenia gravis

Solution:

C

Exercise:

Problem:

Which of the following is an example of a systemic autoimmune disease?

- A. Hashimoto thyroiditis
- B. type I diabetes mellitus
- C. Graves disease
- D. myasthenia gravis

Solution:

D

Fill in the Blank

Exercise:

Problem:

The thyroid-stimulating immunoglobulin that acts like thyroid-stimulating hormone and causes Graves disease is an antibody to the _____.

Solution:

thyroid-stimulating hormone receptor

Organ Transplantation and Rejection

LEARNING OBJECTIVES

- Explain why human leukocyte antigens (HLAs) are important in tissue transplantation
- Explain the types of grafts possible and their potential for interaction with the immune system
- Describe what occurs during graft-versus-host disease (GVHD)

A graft is the transplantation of an organ or tissue to a different location, with the goal of replacing a missing or damaged organ or tissue. Grafts are typically moved without their attachments to the circulatory system and must reestablish these, in addition to the other connections and interactions with their new surrounding tissues. There are different types of grafts depending on the source of the new tissue or organ. Tissues that are transplanted from one genetically distinct individual to another within the same species are called **allografts**. An interesting variant of the allograft is an **isograft**, in which tissue from one twin is transplanted to another. As long as the twins are monozygotic (therefore, essentially genetically identical), the transplanted tissue is virtually never rejected. If tissues are transplanted from one area on an individual to another area on the same individual (e.g., a skin graft on a burn patient), it is known as an **autograft**. If tissues from an animal are transplanted into a human, this is called a **xenograft**.

Transplant Rejection

The different types of grafts described above have varying risks for rejection ([\[link\]](#)). Rejection occurs when the recipient’s immune system recognizes the donor tissue as foreign (non-self), triggering an immune response. The major histocompatibility complex markers MHC I and MHC II, more specifically identified as human leukocyte antigens (HLAs), play a role in transplant rejection. The HLAs expressed in tissue transplanted from a genetically different individual or species may be recognized as non-self molecules by the host’s dendritic cells. If this occurs, the dendritic cells will process and present the foreign HLAs to the host’s helper T cells and cytotoxic T cells, thereby activating them. Cytotoxic T cells then target and kill the grafted cells through the same mechanism they use to kill virus-infected cells; helper T cells may also release cytokines that activate macrophages to kill graft cells.

Types of Tissue and Organ Grafts and Their Complications		
Graft	Procedure	Complications
Autograft	From self to self	No rejection concerns
Isograft	From identical twin to twin	Little concern of rejection
Allograft	From relative or nonrelative to individual	Rejection possible
Xenograft	From animal to human	Rejection possible

With the three highly polymorphic MHC I genes in humans (*HLA-A*, *HLA-B*, and *HLA-C*) determining compatibility, each with many alleles segregating in a population, odds are extremely low that a randomly chosen

donor will match a recipient's six-allele genotype (the two alleles at each locus are expressed codominantly). This is why a parent or a sibling may be the best donor in many situations—a genetic match between the MHC genes is much more likely and the organ is much less likely to be rejected.

Although matching all of the MHC genes can lower the risk for rejection, there are a number of additional gene products that also play a role in stimulating responses against grafted tissue. Because of this, no non-self grafted tissue is likely to completely avoid rejection. However, the more similar the MHC gene match, the more likely the graft is to be tolerated for a longer time. Most transplant recipients, even those with tissues well matched to their MHC genes, require treatment with immunosuppressant drugs for the rest of their lives. This can make them more vulnerable than the general population to complications from infectious diseases. It can also result in transplant-related malignancies because the body's normal defenses against cancer cells are being suppressed.

Note:

- What part of the immune response is responsible for graft rejection?
- Explain why blood relatives are preferred as organ donors.
- Describe the role of immunosuppression in transplantation.

Graft-versus-Host Disease

A form of rejection called **graft-versus-host disease (GVHD)** primarily occurs in recipients of bone marrow transplants and peripheral blood stem cells. GVHD presents a unique situation because the transplanted tissue is capable of producing immune cells; APCs in the donated bone marrow may recognize the host cells as non-self, leading to activation of the donor cytotoxic T cells. Once activated, the donor's T cells attack the recipient cells, causing acute GVHD.

Acute GVHD typically develops within weeks after a bone marrow transplant, causing tissue damage affecting the skin, gastrointestinal tract, liver, and eyes. In addition, acute GVHD may also lead to a cytokine storm, an unregulated secretion of cytokines that may be fatal. In addition to acute GVHD, there is also the risk for chronic GVHD developing months after the bone marrow transplant. The mechanisms responsible for chronic GVHD are not well understood.

To minimize the risk of GVHD, it is critically important to match the HLAs of the host and donor as closely as possible in bone marrow transplants. In addition, the donated bone marrow is processed before grafting to remove as many donor APCs and T cells as possible, leaving mostly hematopoietic stem cells.

Note:

- Why does GVHD occur in specifically in bone marrow transplants?
- What cells are responsible for GVHD?

The Future of Transplantation

Historically speaking, the practice of transplanting tissues—and the complications that can accompany such procedures—is a relatively recent development. It was not until 1954 that the first successful organ transplantation between two humans was achieved. Yet the field of organ transplantation has progressed rapidly since that time.

Nonetheless, the practice of transplanting non-self tissues may soon become obsolete. Scientists are now attempting to develop methods by which new organs may be grown *in vitro* from an individual's own harvested cells to replace damaged or abnormal ones. Because organs produced in this way would contain the individual's own cells, they could be transplanted into the individual without risk for rejection.

An alternative approach that is gaining renewed research interest is genetic modification of donor animals, such as pigs, to provide transplantable organs that do not elicit an immune response in the recipient. The approach involves excising the genes in the pig (in the embryo) that are most responsible for the rejection reaction after transplantation. Finding these genes and effectively removing them is a challenge, however. So too is identifying and neutralizing risks from viral sequences that might be embedded in the pig genome, posing a risk for infection in the human recipient.

Note:

There are currently more than a dozen different tissues and organs used in human transplantations. Learn more about them at [this](#) website.

Note:

Resolution

Kerry's tests come back positive, confirming a diagnosis of lupus, a disease that occurs 10 times more frequently in women than men. SLE cannot be cured, but there are various therapies available for reducing and managing its symptoms. Specific therapies are prescribed based on the particular symptoms presenting in the patient. Kerry's rheumatologist starts her therapy with a low dose of corticosteroids to reduce her rashes. She also prescribes a low dose of hydroxychloroquine, an anti-inflammatory drug that is used to treat inflammation in patients with RA, childhood arthritis, SLE, and other autoimmune diseases. Although the mechanism of action of hydroxychloroquine is not well defined, it appears that this drug interferes with the processes of antigen processing and activation of autoimmunity. Because of its mechanism, the effects of hydroxychloroquine are not as immediate as that of other anti-inflammatory drugs, but it is still considered a good companion therapy for SLE. Kerry's doctor also advises her to limit her exposure to sunlight, because photosensitivity to sunlight may precipitate rashes.

Over the next 6 months, Kerry follows her treatment plan and her symptoms do not return. However, future flare-ups are likely to occur. She will need to continue her treatment for the rest of her life and seek medical attention whenever new symptoms develop.

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Grafts and transplants can be classified as **autografts**, **isografts**, **allografts**, or **xenografts** based on the genetic differences between the donor's and recipient's tissues.
- Genetic differences, especially among the MHC (HLA) genes, will dictate the likelihood that **rejection** of the transplanted tissue will occur.
- Transplant recipients usually require immunosuppressive therapy to avoid rejection, even with good genetic matching. This can create additional problems when immune responses are needed to fight off infectious agents and prevent cancer.
- **Graft-versus-host disease** can occur in bone marrow transplants, as the mature T cells in the transplant itself recognize the recipient's tissues as foreign.
- Transplantation methods and technology have improved greatly in recent decades and may move into new areas with the use of stem cell technology to avoid the need for genetic matching of MHC molecules.

Matching

Exercise:

Problem: Match the graft with its description.

___autograft	A. donor is a different species than the recipient
___allograft	B. donor and recipient are the same individual
___xenograft	C. donor is an identical twin of the recipient
___isograft	D. donor is the same species as the recipient, but genetically different

Solution:

B, D, A, C

Fill in the Blank

Exercise:

Problem:

For a transplant to have the best chances of avoiding rejection, the genes coding for the _____ molecules should be closely matched between donor and recipient.

Solution:

MHC

Exercise:

Problem:

Because it is a “transplant” that can include APCs and T cells from the donor, a bone marrow transplant may induce a very specific type of rejection known as _____ disease.

Solution:

graft-versus-host

Short Answer

Exercise:

Problem:

Why is a parent usually a better match for transplanted tissue to a donor than a random individual of the same species?

Immunodeficiency

LEARNING OBJECTIVES

- Compare the causes of primary and secondary immunodeficiencies
- Describe treatments for primary and secondary immunodeficiencies

Immunodeficiencies are inherited (primary) or acquired (secondary) disorders in which elements of host immune defenses are either absent or functionally defective. In developed countries, most immunodeficiencies are inherited, and they are usually first seen in the clinic as recurrent or overwhelming infections in infants. However, on a global scale, malnutrition is the most common cause of immunodeficiency and would be categorized as an acquired immunodeficiency. Acquired immunodeficiencies are more likely to develop later in life, and the pathogenic mechanisms of many remain obscure.

Primary Immunodeficiency

Primary immunodeficiencies, which number more than 250, are caused by inherited defects of either nonspecific innate or specific adaptive immune defenses. In general, patients born with primary immunodeficiency (PI) commonly have an increased susceptibility to infection. This susceptibility can become apparent shortly after birth or in early childhood for some individuals, whereas other patients develop symptoms later in life. Some primary immunodeficiencies are due to a defect of a single cellular or humoral component of the immune system; others may result from defects of more than one component. Examples of primary immunodeficiencies include chronic granulomatous disease, X-linked agammaglobulinemia, selective IgA deficiency, and severe combined immunodeficiency disease.

Chronic Granulomatous Disease

The causes of **chronic granulomatous disease** (CGD) are defects in the NADPH oxidase system of phagocytic cells, including neutrophils and macrophages, that prevent the production of superoxide radicals in phagolysosomes. The inability to produce superoxide radicals impairs the antibacterial activity of phagocytes. As a result, infections in patients with CGD persist longer, leading to a chronic local inflammation called a granuloma. Microorganisms that are the most common causes of infections in patients with CGD include

Aspergillus spp., *Staphylococcus aureus*, *Chromobacterium violaceum*, *Serratia marcescens*, and *Salmonella typhimurium*.

X-Linked Agammaglobulinemia

Deficiencies in B cells due to defective differentiation lead to a lack of specific antibody production known as **X-linked agammaglobulinemia**. In 1952, Ogden C. Bruton (1908–2003) described the first immunodeficiency in a boy whose immune system failed to produce antibodies. This defect is inherited on the X chromosome and is characterized by the absence of immunoglobulin in the serum; it is called Bruton X-linked agammaglobulinemia (XLA). The defective gene, *BTK*, in XLA is now known to encode a tyrosine kinase called Bruton tyrosine kinase (Btk). In patients whose B cells are unable to produce sufficient amounts of Btk, the B-cell maturation and differentiation halts at the pre-B-cell stage of growth. B-cell maturation and differentiation beyond the pre-B-cell stage of growth is required for immunoglobulin production. Patients who lack antibody production suffer from recurrent infections almost exclusively due to extracellular pathogens that cause pyogenic infections: *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. pyogenes*, and *S. aureus*. Because cell-mediated immunity is not impaired, these patients are not particularly vulnerable to infections caused by viruses or intracellular pathogens.

Selective IgA Deficiency

The most common inherited form of immunoglobulin deficiency is **selective IgA deficiency**, affecting about one in 800 people. Individuals with selective IgA deficiency produce normal levels of IgG and IgM, but are not able to produce secretory IgA. IgA deficiency predisposes these individuals to lung and gastrointestinal infections for which secretory IgA is normally an important defense mechanism. Infections in the lungs and gastrointestinal tract can involve a variety of pathogens, including *H. influenzae*, *S. pneumoniae*, *Moraxella catarrhalis*, *S. aureus*, *Giardia lamblia*, or pathogenic strains of *Escherichia coli*.

Severe Combined Immunodeficiency

Patients who suffer from **severe combined immunodeficiency (SCID)** have B-cell and T-cell defects that impair T-cell dependent antibody responses as well as cell-mediated immune responses. Patients with SCID also cannot develop immunological memory, so vaccines provide them no protection, and live attenuated vaccines (e.g., for varicella-zoster, measles virus, rotavirus, poliovirus) can actually cause the infection they are intended to prevent. The most common form is X-linked SCID, which accounts for nearly 50% of all cases and occurs primarily in males. Patients with SCID are typically diagnosed within the first few months of life after developing severe, often life-threatening, opportunistic infection by *Candida* spp., *Pneumocystis jirovecii*, or pathogenic strains of *E. coli*.

Without treatment, babies with SCID do not typically survive infancy. In some cases, a bone marrow transplant may successfully correct the defects in lymphocyte development that lead to the SCID phenotype, by replacing the defective component. However, this treatment approach is not without risks, as demonstrated by the famous case of David Vetter (1971–1984), better known as “Bubble Boy” ([link](#)). Vetter, a patient with SCID who lived in a protective plastic bubble to prevent exposure to opportunistic microbes, received a bone marrow transplant from his sister. Because of a latent Epstein-Barr virus infection in her bone marrow, however, he developed mononucleosis and died of Burkitt lymphoma at the age of 12 years.



David Vetter, popularly known as “The Bubble Boy,” was born with SCID and lived most of his life isolated inside a plastic bubble. Here he is shown outside the bubble in a suit specially built for him by NASA. (credit: NASA Johnson Space Center)

Note:

- What is the fundamental cause of a primary immunodeficiency?
- Explain why patients with chronic granulomatous disease are especially susceptible to bacterial infections.
- Explain why individuals with selective IgA deficiency are susceptible to respiratory and gastrointestinal infections.

Secondary Immunodeficiency

A **secondary immunodeficiency** occurs as a result of an acquired impairment of function of B cells, T cells, or both. Secondary immunodeficiencies can be caused by:

- Systemic disorders such as diabetes mellitus, malnutrition, hepatitis, or HIV infection
- Immunosuppressive treatments such as cytotoxic chemotherapy, bone marrow ablation before transplantation, or radiation therapy
- Prolonged critical illness due to infection, surgery, or trauma in the very young, elderly, or hospitalized patients

Unlike primary immunodeficiencies, which have a genetic basis, secondary immunodeficiencies are often reversible if the underlying cause is resolved. Patients with secondary immunodeficiencies develop an increased susceptibility to an otherwise benign infection by opportunistic pathogens such as *Candida* spp., *P. jirovecii*, and *Cryptosporidium*.

HIV infection and the associated **acquired immunodeficiency syndrome (AIDS)** are the best-known secondary immunodeficiencies. AIDS is characterized by profound CD4 T-cell lymphopenia (decrease in lymphocytes). The decrease in CD4 T cells is the result of various mechanisms, including HIV-induced pyroptosis (a type of apoptosis that stimulates an inflammatory response), viral cytopathic effect, and cytotoxicity to HIV-infected cells.

The most common cause of secondary immunodeficiency worldwide is severe malnutrition, which affects both innate and adaptive immunity. More research and information are needed for the more common causes of secondary immunodeficiency; however, the number of new discoveries in AIDS research far exceeds that of any other single cause of secondary immunodeficiency. AIDS research has paid off extremely well in terms of discoveries and treatments; increased research into the most common cause of immunodeficiency, malnutrition, would likely be as beneficial.

Note:

- What is the most common cause of secondary immunodeficiencies?
- Explain why secondary immunodeficiencies can sometimes be reversed.

Note:**An Immunocompromised Host**

Benjamin, a 50-year-old male patient who has been receiving chemotherapy to treat his chronic myelogenous leukemia (CML), a disease characterized by massive overproduction of nonfunctional, malignant myelocytic leukocytes that crowd out other, healthy leukocytes, is seen in the emergency department. He is complaining of a productive, wet cough, dyspnea, and fatigue. On examination, his pulse is 120 beats per minute (bpm) (normal range is 60–100 bpm) and weak, and his blood pressure is 90/60 mm Hg (normal is 120/80 mm Hg). During auscultation, a distinct crackling can be heard in his lungs as he breathes, and his pulse-oximeter level (a measurement of blood-oxygen saturation) is 80% (normal is 95%–100%). He has a fever; his temperature is 38.9 °C (102 °F). Sputum cultures and blood samples are obtained and sent to the lab, but Benjamin goes into respiratory distress and dies before the results can be obtained.

Benjamin's death was a result of a combination of his immune system being compromised by his leukemia and his chemotherapy treatment further weakening his ability to mount an immune response. CML (and leukemia in general) and corresponding chemotherapy cause a decrease in the number of leukocytes capable of normal function, leading to secondary immunodeficiency. This increases the risk for opportunistic bacterial, viral, protozoal, and fungal infections that could include *Staphylococcus*, enteroviruses, *Pneumocystis*, *Giardia*, or *Candida*. Benjamin's symptoms were suggestive of bacterial pneumonia, but his leukemia and chemotherapy likely complicated and contributed to the severity of the pneumonia, resulting in his death. Because his leukemia was overproducing certain white blood cells, and those overproduced white blood cells were largely nonfunctional or abnormal in their function, he did not have the proper immune system blood cells to help him fight off the infection.

[\[link\]](#) summarizes primary and secondary immunodeficiencies, their effects on immune function, and typical outcomes.

Primary and Secondary Immunodeficiencies		
Disease	Effect on Immune Function	Outcomes

Primary and Secondary Immunodeficiencies			
Disease		Effect on Immune Function	Outcomes
Primary immunodeficiencies	Chronic granulomatous disease	Impaired killing of bacteria within the phagolysosome of neutrophils and macrophages	Chronic infections and granulomas
	Selective IgA deficiency	Inability to produce secretory IgA	Predisposition to lung and gastrointestinal infections
	Severe combined immunodeficiency disease (SCID)	Deficient humoral and cell-mediated immune responses	Early development of severe and life-threatening opportunistic infections
	X-linked agammaglobulinemia	Flawed differentiation of B cells and absence of specific antibodies	Recurrent infections almost exclusively due to pathogens that cause pyogenic infections
Secondary immunodeficiencies	Immunosuppressive therapies (e.g., chemotherapy, radiotherapy)	Impaired humoral and/or cell-mediated immune responses	Opportunistic infections, rare cancers

Primary and Secondary Immunodeficiencies			
Disease		Effect on Immune Function	Outcomes
	Malnutrition	Impaired humoral and/or cell-mediated immune responses	Opportunistic infections, rare cancers
	Viral infection (e.g., HIV)	Impaired cell-mediated immune responses due to CD4 T-cell lymphopenia	Opportunistic infections, rare cancers

Key Concepts and Summary

- **Primary immunodeficiencies** are caused by genetic abnormalities; **secondary immunodeficiencies** are acquired through disease, diet, or environmental exposures
- Primary immunodeficiencies may result from flaws in phagocyte killing of innate immunity, or impairment of T cells and B cells.
- Primary immunodeficiencies include chronic granulomatous disease, X-linked agammaglobulinemia, selective IgA deficiency, and severe combined immunodeficiency disease.
- Secondary immunodeficiencies result from environmentally induced defects in B cells and/or T cells.
- Causes for secondary immunodeficiencies include malnutrition, viral infection, diabetes, prolonged infections, and chemical or radiation exposure.

Multiple Choice

Exercise:

Problem:

Which of the following is a genetic disease that results in lack of production of antibodies?

- A. agammaglobulinemia
- B. myasthenia gravis
- C. HIV/AIDS

D. chronic granulomatous disease

Solution:

A

Exercise:

Problem:

Which of the following is a genetic disease that results in almost no adaptive immunity due to lack of B and/ or T cells?

- A. agammaglobulinemia
- B. severe combined immunodeficiency
- C. HIV/AIDS
- D. chronic granulomatous disease

Solution:

B

Exercise:

Problem:

All but which one of the following are examples of secondary immunodeficiencies?

- A. HIV/AIDS
- B. malnutrition
- C. chronic granulomatous disease
- D. immunosuppression due to measles infection

Solution:

C

Fill in the Blank

Exercise:

Problem:

Diseases due to _____ abnormalities are termed primary immunodeficiencies.

Solution:

genetic

Exercise:

Problem: A secondary immunodeficiency is _____, rather than genetic.

Solution:

acquired

Short Answer

Exercise:

Problem: Compare the treatments for primary and secondary immunodeficiencies.

LEARNING OBJECTIVES

- Explain how the adaptive specific immune response responds to tumors
- Discuss the risks and benefits of tumor vaccines

Cancer involves a loss of the ability of cells to control their cell cycle, the stages each eukaryotic cell goes through as it grows and then divides. When this control is lost, the affected cells rapidly divide and often lose the ability to differentiate into the cell type appropriate for their location in the body. In addition, they lose contact inhibition and can start to grow on top of each other. This can result in formation of a **tumor**. It is important to make a distinction here: The term “cancer” is used to describe the diseases resulting from loss of cell-cycle regulation and subsequent cell proliferation. But the term “tumor” is more general. A “tumor” is an abnormal mass of cells, and a tumor can be benign (not cancerous) or malignant (cancerous).

Traditional cancer treatment uses radiation and/or chemotherapy to destroy cancer cells; however, these treatments can have unwanted side effects because they harm normal cells as well as cancer cells. Newer, promising therapies attempt to enlist the patient’s immune system to target cancer cells specifically. It is known that the immune system can recognize and destroy cancerous cells, and some researchers and immunologists also believe, based on the results of their experiments, that many cancers are eliminated by the body’s own defenses before they can become a health problem. This idea is not universally accepted by researchers, however, and needs further investigation for verification.

Cell-Mediated Response to Tumors

Cell-mediated immune responses can be directed against cancer cells, many of which do not have the normal complement of self-proteins, making them a target for elimination. Abnormal cancer cells may also present tumor antigens. These tumor antigens are not a part of the screening process used to eliminate lymphocytes during development; thus, even though they are self-antigens, they can stimulate and drive adaptive immune responses against abnormal cells.

Presentation of tumor antigens can stimulate naïve helper T cells to become activated by cytokines such as IL-12 and differentiate into T_H1 cells. T_H1 cells release cytokines that can activate natural killer (NK) cells and enhance the killing of activated cytotoxic T cells. Both NK cells and cytotoxic T cells can recognize and target cancer cells, and induce apoptosis through the action of perforins and granzymes. In addition, activated cytotoxic T cells can bind to cell-surface proteins on abnormal cells and induce apoptosis by a second killing mechanism called the CD95 (Fas) cytotoxic pathway.

Despite these mechanisms for removing cancerous cells from the body, cancer remains a common cause of death. Unfortunately, malignant tumors tend to actively suppress the immune response in various ways. In some cancers, the immune cells themselves are cancerous. In leukemia, lymphocytes that would normally facilitate the immune response become abnormal. In other cancers, the cancerous cells can become resistant to induction of apoptosis. This may occur through the expression of membrane proteins that shut off cytotoxic T cells or that induce regulatory T cells that can shut down immune responses.

The mechanisms by which cancer cells alter immune responses are still not yet fully understood, and this is a very active area of research. As scientists' understanding of adaptive immunity improves, cancer therapies that harness the body's immune defenses may someday be more successful in treating and eliminating cancer.

Note:

- How do cancer cells suppress the immune system?
- Describe how the immune system recognizes and destroys cancer cells.

Cancer Vaccines

There are two types of cancer vaccines: preventive and therapeutic. Preventive vaccines are used to prevent cancer from occurring, whereas therapeutic vaccines are used to treat patients with cancer. Most preventive cancer vaccines target viral infections that are known to lead to cancer. These include vaccines against human papillomavirus (HPV) and hepatitis B, which help prevent cervical and liver cancer, respectively.

Most therapeutic cancer vaccines are in the experimental stage. They exploit tumor-specific antigens to stimulate the immune system to selectively attack cancer cells. Specifically, they aim to enhance T_H1 function and interaction with cytotoxic T cells, which, in turn, results in more effective attack on abnormal tumor cells. In some cases, researchers have used genetic engineering to develop antitumor vaccines in an approach similar to that used for DNA vaccines (see [Micro Connections: DNA vaccines](#)). The vaccine contains a recombinant plasmid with genes for tumor antigens; theoretically, the tumor gene would not induce new cancer because it is not functional, but it could trick the immune system into targeting the tumor gene product as a foreign invader.

The first FDA-approved therapeutic cancer vaccine was sipuleucel-T (Provenge), approved in 2010 to treat certain cases of prostate cancer. [\[footnote\]](#) This unconventional vaccine is custom designed using the patient's own cells. APCs are removed from the patient and cultured with a tumor-specific molecule; the cells are then returned to the patient. This approach appears to enhance the patient's immune response against the cancer cells. Another therapeutic cancer vaccine (talimogene laherparepvec, also called T-VEC or Imlygic) was approved by the FDA in 2015 for

treatment of melanoma, a form of skin cancer. This vaccine contains a virus that is injected into tumors, where it infects and lyses the tumor cells. The virus also induces a response in lesions or tumors besides those into which the vaccine is injected, indicating that it is stimulating a more general (as opposed to local) antitumor immune response in the patient.

National Institutes of Health, National Cancer Institute. "Cancer Vaccines." <http://www.cancer.gov/about-cancer/causes-prevention/vaccines-fact-sheet#q8>. Accessed on May 20, 2016.

Note:

- Explain the difference between preventative and therapeutic cancer vaccines.
- Describe at least two different approaches to developing therapeutic anti-cancer vaccines.

Note:

Using Viruses to Cure Cancer

Viruses typically destroy the cells they infect—a fact responsible for any number of human diseases. But the cell-killing powers of viruses may yet prove to be the cure for some types of cancer, which is generally treated by attempting to rid the body of cancerous cells. Several clinical trials are studying the effects of viruses targeted at cancer cells. Reolysin, a drug currently in testing phases, uses reoviruses (respiratory enteric orphan viruses) that can infect and kill cells that have an activated Ras-signaling pathway, a common mutation in cancerous cells. Viruses such as rubeola (the measles virus) can also be genetically engineered to aggressively attack tumor cells. These modified viruses not only bind more specifically to receptors overexpressed on cancer cells, they also carry genes driven by promoters that are only turned on within cancer cells. Herpesvirus and others have also been modified in this way.

Key Concepts and Summary

- Cancer results from a loss of control of the cell cycle, resulting in uncontrolled cell proliferation and a loss of the ability to differentiate.
- Adaptive and innate immune responses are engaged by **tumor** antigens, self-molecules only found on abnormal cells. These adaptive responses stimulate helper T cells to activate cytotoxic T cells and NK cells of innate immunity that will seek and destroy cancer cells.
- New anticancer therapies are in development that will exploit natural adaptive immunity anticancer responses. These include external stimulation of cytotoxic T cells and therapeutic vaccines that assist or enhance the immune response.

Multiple Choice

Exercise:

Problem:

Cancer results when a mutation leads to which of the following?

- A. cell death
- B. apoptosis
- C. loss of cell-cycle control
- D. shutdown of the cell cycle

Solution:

C

Exercise:

Problem:

Tumor antigens are _____ that are inappropriately expressed and found on abnormal cells.

- A. self antigens

- B. foreign antigens
- C. antibodies
- D. T-cell receptors

Solution:

A

Fill in the Blank

Exercise:

Problem:

A _____ cancer vaccine is one that stops the disease from occurring in the first place.

Solution:

preventive

Exercise:

Problem:

A _____ cancer vaccine is one that will help to treat the disease after it has occurred.

Solution:

therapeutic

Short Answer

Exercise:

Problem:

How can tumor antigens be effectively targeted without inducing an autoimmune (anti-self) response?

Introduction

class="introduction"

The skin is an important barrier to pathogens, but it can also develop infections.

These raised lesions (left) are typical of folliculitis, a condition that results from the inflammation of hair follicles.

Acne lesions (right) also result from inflammation of hair follicles. In this case, the inflammation results when hair follicles become clogged with complex lipids, fatty acids, and dead skin

cells,
producing a
favorable
environment
for bacteria.



The human body is covered in skin, and like most coverings, skin is designed to protect what is underneath. One of its primary purposes is to prevent microbes in the surrounding environment from invading underlying tissues and organs. But in spite of its role as a protective covering, skin is not itself immune from infection. Certain pathogens and toxins can cause severe infections or reactions when they come in contact with the skin. Other pathogens are opportunistic, breaching the skin's natural defenses through cuts, wounds, or a disruption of normal microbiota resulting in an infection in the surrounding skin and tissue. Still other pathogens enter the body via different routes—through the respiratory or digestive systems, for example—but cause reactions that manifest as skin rashes or lesions.

Nearly all humans experience skin infections to some degree. Many of these conditions are, as the name suggests, “skin deep,” with symptoms that are local and non-life-threatening. At some point, almost everyone must endure conditions like acne, athlete’s foot, and minor infections of cuts and abrasions, all of which result from infections of the skin. But not all skin infections are quite so innocuous. Some can become invasive, leading to

systemic infection or spreading over large areas of skin, potentially becoming life-threatening.

Anatomy and Normal Microbiota of the Skin and Eyes

LEARNING OBJECTIVES

- Describe the major anatomical features of the skin and eyes
- Compare and contrast the microbiomes of various body sites, such as the hands, back, feet, and eyes
- Explain how microorganisms overcome defenses of skin and eyes in order to cause infection
- Describe general signs and symptoms of disease associated with infections of the skin and eyes

Note:

Part 1

Sam, a college freshman with a bad habit of oversleeping, nicked himself shaving in a rush to get to class on time. At the time, he didn't think twice about it. But two days later, he noticed the cut was surrounded by a reddish area of skin that was warm to the touch. When the wound started oozing pus, he decided he had better stop by the university's clinic. The doctor took a sample from the lesion and then cleaned the area.

- What type of microbe could be responsible for Sam's infection?

Jump to the [next](#) Clinical Focus box.

Human skin is an important part of the innate immune system. In addition to serving a wide range of other functions, the skin serves as an important

barrier to microbial invasion. Not only is it a physical barrier to penetration of deeper tissues by potential pathogens, but it also provides an inhospitable environment for the growth of many pathogens. In this section, we will provide a brief overview of the anatomy and normal microbiota of the skin and eyes, along with general symptoms associated with skin and eye infections.

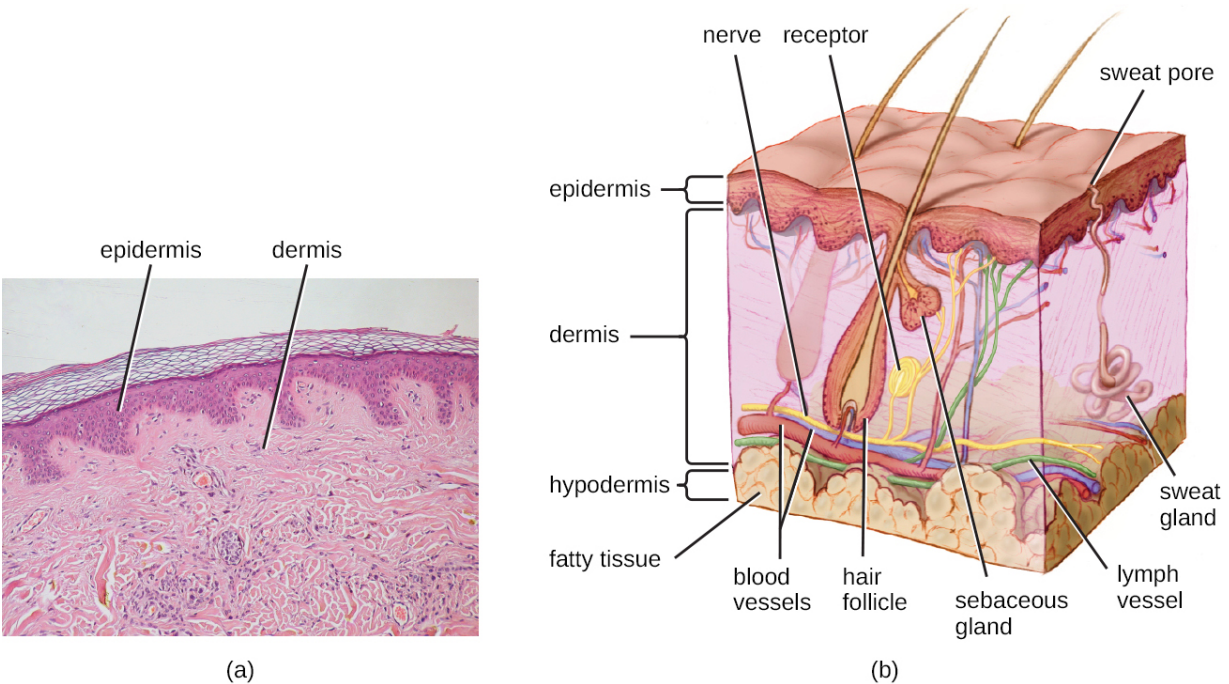
Layers of the Skin

Human skin is made up of several layers and sublayers. The two main layers are the **epidermis** and the **dermis**. These layers cover a third layer of tissue called the **hypodermis**, which consists of fibrous and adipose connective tissue ([link](#)).

The epidermis is the outermost layer of the skin, and it is relatively thin. The exterior surface of the epidermis, called the **stratum corneum**, primarily consists of dead skin cells. This layer of dead cells limits direct contact between the outside world and live cells. The stratum corneum is rich in **keratin**, a tough, fibrous protein that is also found in hair and nails. Keratin helps make the outer surface of the skin relatively tough and waterproof. It also helps to keep the surface of the skin dry, which reduces microbial growth. However, some microbes are still able to live on the surface of the skin, and some of these can be shed with dead skin cells in the process of **desquamation**, which is the shedding and peeling of skin that occurs as a normal process but that may be accelerated when infection is present.

Beneath the epidermis lies a thicker skin layer called the dermis. The dermis contains connective tissue and embedded structures such as blood vessels, nerves, and muscles. Structures called **hair follicles** (from which hair grows) are located within the dermis, even though much of their structure consists of epidermal tissue. The dermis also contains the two major types of glands found in human skin: **sweat glands** (tubular glands that produce sweat) and **sebaceous glands** (which are associated with hair follicles and produce **sebum**, a lipid-rich substance containing proteins and minerals).

Perspiration (sweat) provides some moisture to the epidermis, which can increase the potential for microbial growth. For this reason, more microbes are found on the regions of the skin that produce the most sweat, such as the skin of the underarms and groin. However, in addition to water, sweat also contains substances that inhibit microbial growth, such as salts, lysozyme, and antimicrobial peptides. Sebum also serves to protect the skin and reduce water loss. Although some of the lipids and fatty acids in sebum inhibit microbial growth, sebum contains compounds that provide nutrition for certain microbes.



(a) A micrograph of a section through human skin shows the epidermis and dermis. (b) The major layers of human skin are the epidermis, dermis, and hypodermis. (credit b: modification of work by National Cancer Institute)

Note:

- How does desquamation help with preventing infections?

Normal Microbiota of the Skin

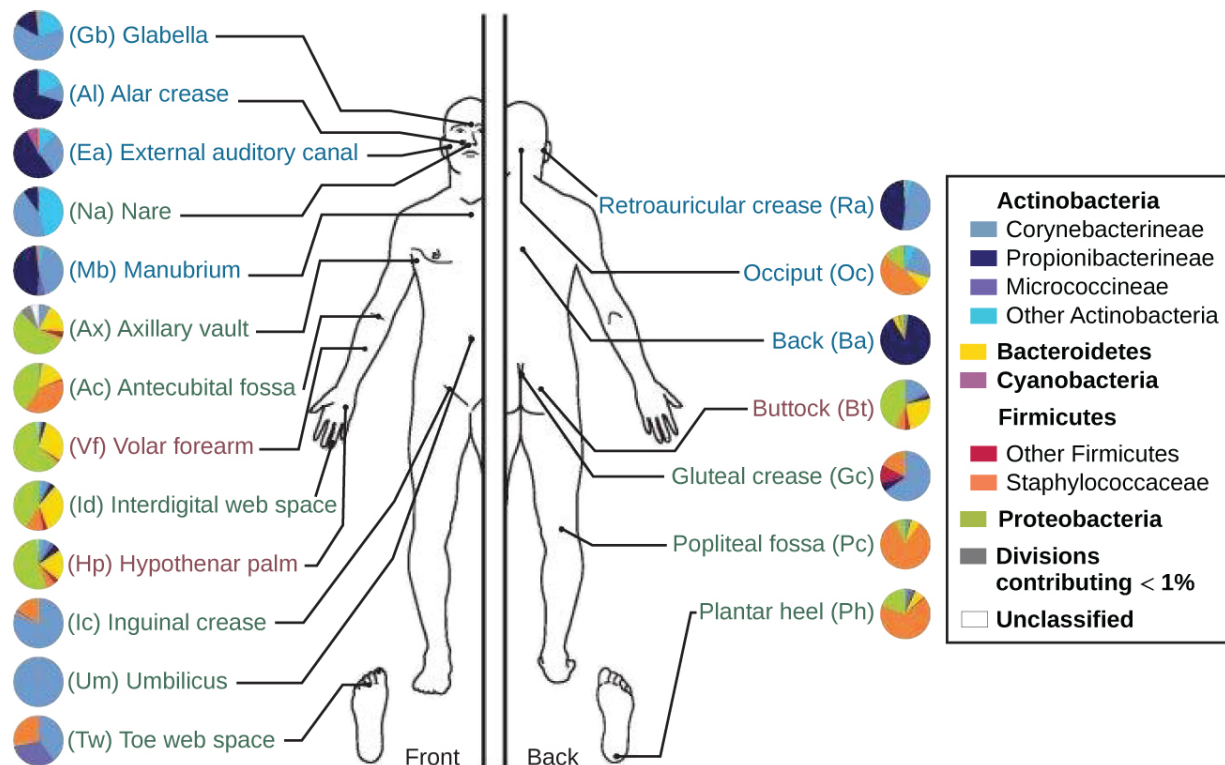
The skin is home to a wide variety of normal microbiota, consisting of commensal organisms that derive nutrition from skin cells and secretions such as sweat and sebum. The normal microbiota of skin tends to inhibit transient-microbe colonization by producing antimicrobial substances and outcompeting other microbes that land on the surface of the skin. This helps to protect the skin from pathogenic infection.

The skin's properties differ from one region of the body to another, as does the composition of the skin's microbiota. The availability of nutrients and moisture partly dictates which microorganisms will thrive in a particular region of the skin. Relatively moist skin, such as that of the nares (nostrils) and underarms, has a much different microbiota than the dryer skin on the arms, legs, hands, and top of the feet. Some areas of the skin have higher densities of sebaceous glands. These sebum-rich areas, which include the back, the folds at the side of the nose, and the back of the neck, harbor distinct microbial communities that are less diverse than those found on other parts of the body.

Different types of bacteria dominate the dry, moist, and sebum-rich regions of the skin. The most abundant microbes typically found in the dry and sebaceous regions are Betaproteobacteria and Propionibacteria, respectively. In the moist regions, *Corynebacterium* and *Staphylococcus* are most commonly found ([\[link\]](#)). Viruses and fungi are also found on the skin, with *Malassezia* being the most common type of fungus found as part of the normal microbiota. The role and populations of viruses in the microbiota, known as viromes, are still not well understood, and there are limitations to the techniques used to identify them. However, Circoviridae, Papillomaviridae, and Polyomaviridae appear to be the most common residents in the healthy skin virome.[\[footnote\]](#)[\[footnote\]](#)[\[footnote\]](#)
Belkaid, Y., and J.A. Segre. "Dialogue Between Skin Microbiota and Immunity," *Science* 346 (2014) 6212:954–959.

Foulongne, Vincent, et al. "Human Skin Microbiota: High Diversity of DNA Viruses Identified on the Human Skin by High Throughput Sequencing." *PLoS ONE* (2012) 7(6): e38499. doi: 10.1371/journal.pone.0038499.

Robinson, C.M., and J.K. Pfeiffer. "Viruses and the Microbiota." *Annual Review of Virology* (2014) 1:55–59. doi: 10.1146/annurev-virology-031413-085550.



The normal microbiota varies on different regions of the skin, especially in dry versus moist areas. The figure shows the major organisms commonly found in different locations of a healthy individual's skin and external mucosa. Note that there is significant variation among individuals. (credit: modification of work by National Human Genome Research Institute)

Note:

- What are the four most common bacteria that are part of the normal skin microbiota?

Infections of the Skin

While the microbiota of the skin can play a protective role, it can also cause harm in certain cases. Often, an opportunistic pathogen residing in the skin microbiota of one individual may be transmitted to another individual more susceptible to an infection. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) can often take up residence in the nares of health care workers and hospital patients; though harmless on intact, healthy skin, MRSA can cause infections if introduced into other parts of the body, as might occur during surgery or via a post-surgical incision or wound. This is one reason why clean surgical sites are so important.

Injury or damage to the skin can allow microbes to enter deeper tissues, where nutrients are more abundant and the environment is more conducive to bacterial growth. Wound infections are common after a puncture or laceration that damages the physical barrier of the skin. Microbes may infect structures in the dermis, such as hair follicles and glands, causing a localized infection, or they may reach the bloodstream, which can lead to a systemic infection.

In some cases, infectious microbes can cause a variety of rashes or lesions that differ in their physical characteristics. These rashes can be the result of inflammation reactions or direct responses to toxins produced by the microbes. [\[link\]](#) lists some of the medical terminology used to describe skin lesions and rashes based on their characteristics; [\[link\]](#) and [\[link\]](#) illustrate some of the various types of skin lesions. It is important to note that many different diseases can lead to skin conditions of very similar appearance; thus the terms used in the table are generally not exclusive to a particular type of infection or disease.

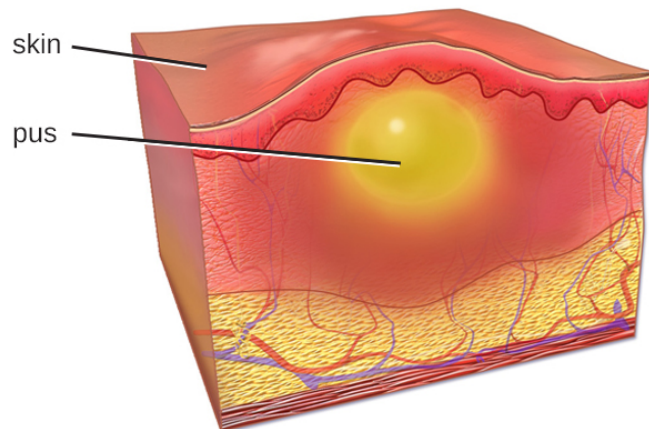
Some Medical Terms Associated with Skin Lesions and Rashes

Term	Definition
abscess	localized collection of pus
bulla (pl., bullae)	fluid-filled blister no more than 5 mm in diameter
carbuncle	deep, pus-filled abscess generally formed from multiple furuncles
crust	dried fluids from a lesion on the surface of the skin
cyst	encapsulated sac filled with fluid, semi-solid matter, or gas, typically located just below the upper layers of skin
folliculitis	a localized rash due to inflammation of hair follicles
furuncle (boil)	pus-filled abscess due to infection of a hair follicle
macules	smooth spots of discoloration on the skin
papules	small raised bumps on the skin
pseudocyst	lesion that resembles a cyst but with a less defined boundary
purulent	pus-producing; suppurative
pustules	fluid- or pus-filled bumps on the skin
pyoderma	any suppurative (pus-producing) infection of the skin

Some Medical Terms Associated with Skin Lesions and Rashes	
Term	Definition
suppurative	producing pus; purulent
ulcer	break in the skin; open sore
vesicle	small, fluid-filled lesion
wheel	swollen, inflamed skin that itches or burns, such as from an insect bite



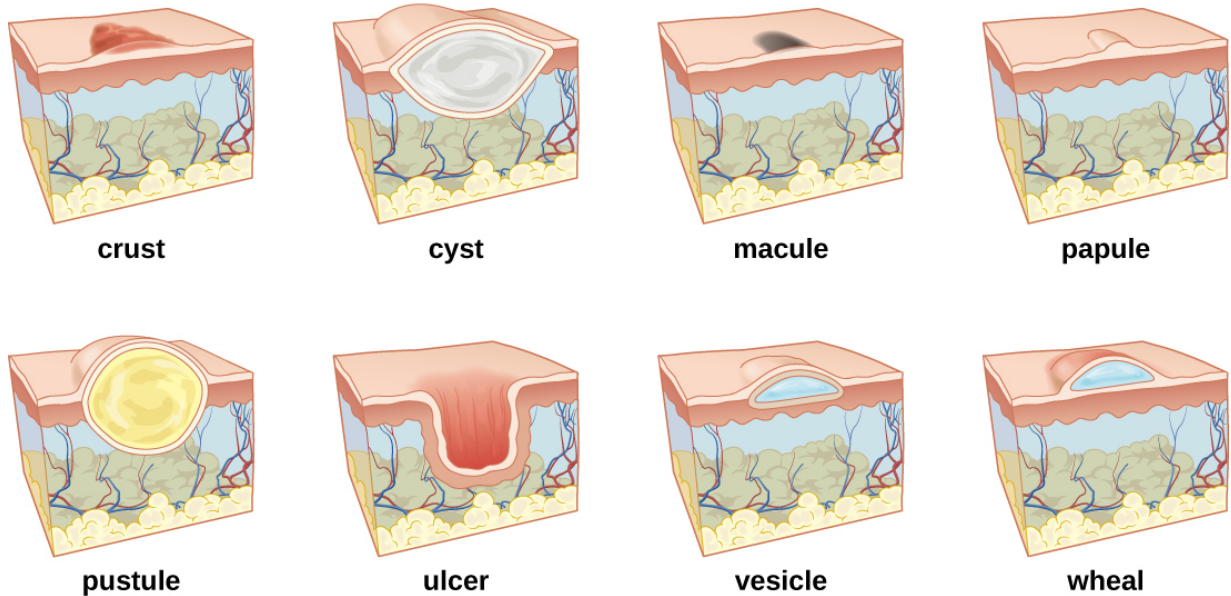
(a)



(b)

(a) Acne is a bacterial infection of the skin that manifests as a rash of inflamed hair follicles (folliculitis). The large whitehead near the center of the cheek is an infected hair follicle that has become purulent (or suppurative), leading to the formation of a furuncle. (b) An abscess is a pus-filled lesion. (credit b: modification of work by Bruce Blaus)

Types of Skin Lesions



Numerous causes can lead to skin lesions of various types, some of which are very similar in appearance. (credit: modification of work by Bruce Blaus)

Note:

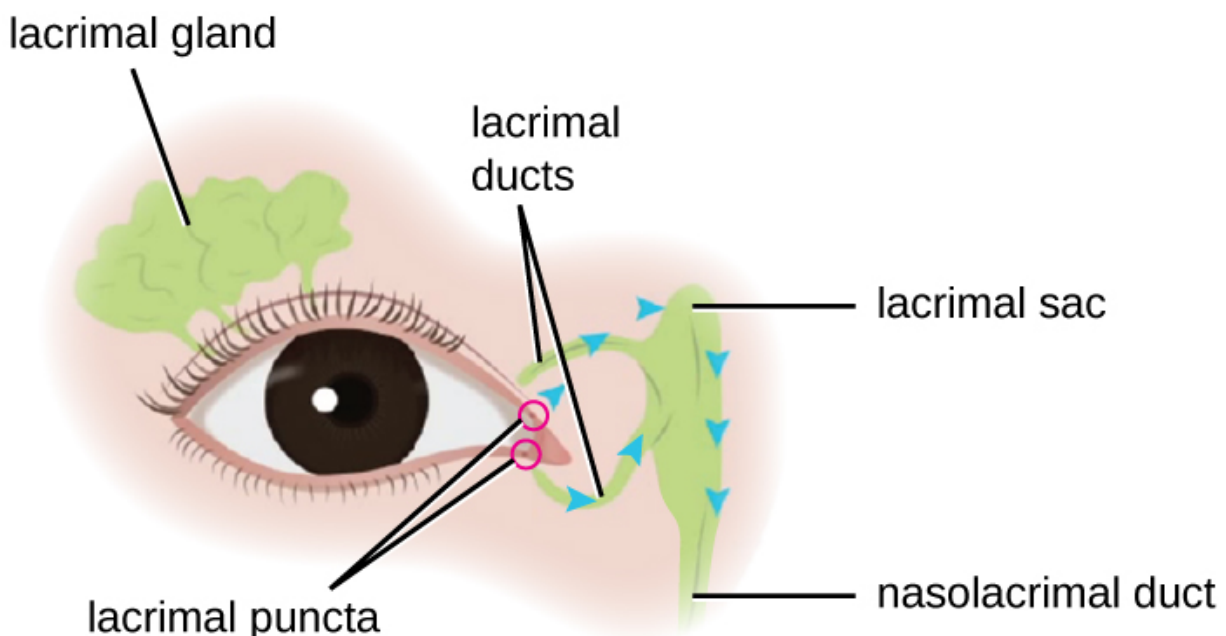
- How can asymptomatic health care workers transmit bacteria such as MRSA to patients?

Anatomy and Microbiota of the Eye

Although the eye and skin have distinct anatomy, they are both in direct contact with the external environment. An important component of the eye is the nasolacrimal drainage system, which serves as a conduit for the fluid of the eye, called tears. Tears flow from the external eye to the nasal cavity

by the lacrimal apparatus, which is composed of the structures involved in tear production ([link](#)). The **lacrimal gland**, above the eye, secretes tears to keep the eye moist. There are two small openings, one on the inside edge of the upper eyelid and one on the inside edge of the lower eyelid, near the nose. Each of these openings is called a **lacrimal punctum**. Together, these lacrimal puncta collect tears from the eye that are then conveyed through **lacrimal ducts** to a reservoir for tears called the **lacrimal sac**, also known as the dacryocyst or tear sac.

From the sac, tear fluid flows via a **nasolacrimal duct** to the inner nose. Each nasolacrimal duct is located underneath the skin and passes through the bones of the face into the nose. Chemicals in tears, such as defensins, lactoferrin, and lysozyme, help to prevent colonization by pathogens. In addition, mucins facilitate removal of microbes from the surface of the eye.



The lacrimal apparatus includes the structures of the eye associated with tear production and drainage. (credit: modification of work by “Evidence Based Medical Educator Inc.”/YouTube)

The surfaces of the eyeball and inner eyelid are mucous membranes called **conjunctiva**. The normal conjunctival microbiota has not been well characterized, but does exist. One small study (part of the Ocular Microbiome project) found twelve genera that were consistently present in the conjunctiva.[\[footnote\]](#) These microbes are thought to help defend the membranes against pathogens. However, it is still unclear which microbes may be transient and which may form a stable microbiota.[\[footnote\]](#)

Abelson, M.B., Lane, K., and Slocum, C.. “The Secrets of Ocular Microbiomes.” *Review of Ophthalmology* June 8, 2015.

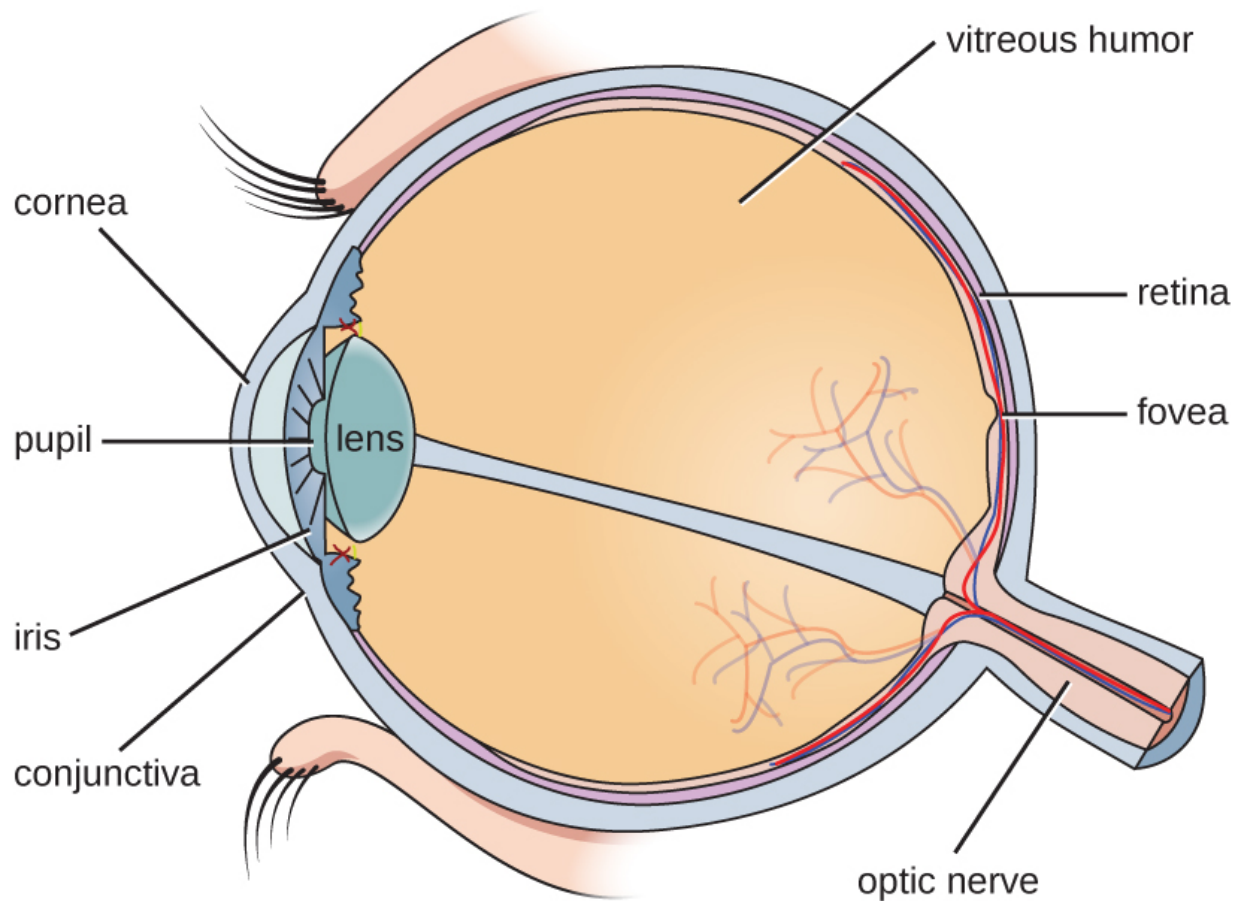
http://www.reviewofophthalmology.com/content/t/ocular_disease/c/55178. Accessed Sept 14, 2016.

Shaikh-Lesko, R. “Visualizing the Ocular Microbiome.” *The Scientist* May 12, 2014. [http://www.the-scientist.com/?](http://www.the-scientist.com/?articles.view/articleNo/39945/title/Visualizing-the-Ocular-Microbiome)

[articles.view/articleNo/39945/title/Visualizing-the-Ocular-Microbiome](http://www.the-scientist.com/?articles.view/articleNo/39945/title/Visualizing-the-Ocular-Microbiome). Accessed Sept 14, 2016.

Use of contact lenses can cause changes in the normal microbiota of the conjunctiva by introducing another surface into the natural anatomy of the eye. Research is currently underway to better understand how contact lenses may impact the normal microbiota and contribute to eye disease.

The watery material inside of the eyeball is called the vitreous humor. Unlike the conjunctiva, it is protected from contact with the environment and is almost always sterile, with no normal microbiota ([\[link\]](#)).



Some microbes live on the conjunctiva of the human eye, but the vitreous humor is sterile.

Infections of the Eye

The conjunctiva is a frequent site of infection of the eye; like other mucous membranes, it is also a common portal of entry for pathogens. Inflammation of the conjunctiva is called **conjunctivitis**, although it is commonly known as pinkeye because of the pink appearance in the eye. Infections of deeper structures, beneath the cornea, are less common ([\[link\]](#)). Conjunctivitis occurs in multiple forms. It may be acute or chronic. Acute purulent conjunctivitis is associated with pus formation, while acute hemorrhagic conjunctivitis is associated with bleeding in the conjunctiva. The term **blepharitis** refers to an inflammation of the eyelids, while **keratitis** refers

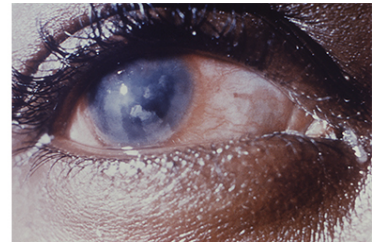
to an inflammation of the cornea ([\[link\]](#)); **keratoconjunctivitis** is an inflammation of both the cornea and the conjunctiva, and **dacryocystitis** is an inflammation of the lacrimal sac that can often occur when a nasolacrimal duct is blocked.



(a)



(b)



(c)

(a) Conjunctivitis is inflammation of the conjunctiva. (b) Blepharitis is inflammation of the eyelids. (c) Keratitis is inflammation of the cornea. (credit a: modification of work by Lopez-Prats MJ, Sanz Marco E, Hidalgo-Mora JJ, Garcia-Delpech S, Diaz-Llopis M; credit b, c: modification of work by Centers for Disease Control and Prevention)

Infections leading to conjunctivitis, blepharitis, keratoconjunctivitis, or dacryocystitis may be caused by bacteria or viruses, but allergens, pollutants, or chemicals can also irritate the eye and cause inflammation of various structures. Viral infection is a more likely cause of conjunctivitis in cases with symptoms such as fever and watery discharge that occurs with upper respiratory infection and itchy eyes. [\[link\]](#) summarizes some common forms of conjunctivitis and blepharitis.

Types of Conjunctivities and Blepharitis

Types of Conjunctivitis and Blepharitis Condition	Description	Causative Agent(s)
Condition	Description	Causative Agent(s)
Acute purulent conjunctivitis	Conjunctivitis with purulent discharge	Bacterial (<i>Haemophilus</i> , <i>Staphylococcus</i>)
Acute hemorrhagic conjunctivitis	Involves subconjunctival hemorrhages	Viral (Picornaviridae)
Acute ulcerative blepharitis	Infection involving eyelids; pustules and ulcers may develop	Bacterial (<i>Staphylococcal</i>) or viral (herpes simplex, varicella-zoster, etc.)
Follicular conjunctivitis	Inflammation of the conjunctiva with nodules (dome-shaped structures that are red at the base and pale on top)	Viral (adenovirus and others); environmental irritants
Dacryocystitis	Inflammation of the lacrimal sac often associated with a plugged nasolacrimal duct	Bacterial (<i>Haemophilus</i> , <i>Staphylococcus</i> , <i>Streptococcus</i>)

Types of Conjunctivities and Blepharitis

Condition	Description	Causative Agent(s)
Keratitis	Inflammation of cornea	Bacterial, viral, or protozoal; environmental irritants
Keratoconjunctivitis	Inflammation of cornea and conjunctiva	Bacterial, viral (adenoviruses), or other causes (including dryness of the eye)
Nonulcerative blepharitis	Inflammation, irritation, redness of the eyelids without ulceration	Environmental irritants; allergens
Papillary conjunctivitis	Inflammation of the conjunctiva; nodules and papillae with red tops develop	Environmental irritants; allergens

Note:

- How does the lacrimal apparatus help to prevent eye infections?

Key Concepts and Summary

- Human skin consists of two main layers, the **epidermis** and **dermis**, which are situated on top of the **hypodermis**, a layer of connective tissue.
- The skin is an effective physical barrier against microbial invasion.
- The skin's relatively dry environment and normal microbiota discourage colonization by transient microbes.
- The skin's normal microbiota varies from one region of the body to another.
- The **conjunctiva** of the eye is a frequent site for microbial infection, but deeper eye infections are less common; multiple types of conjunctivitis exist.

Multiple Choice

Exercise:

Problem:

_____ glands produce a lipid-rich substance that contains proteins and minerals and protects the skin.

- a. Sweat
- b. Mammary
- c. Sebaceous
- d. Endocrine

Solution:

C

Exercise:

Problem:

Which layer of skin contains living cells, is vascularized, and lies directly above the hypodermis?

- a. the stratum corneum
- b. the dermis
- c. the epidermis
- d. the conjunctiva

Solution:

B

Fill in the Blank

Exercise:

Problem: The _____ is the outermost layer of the epidermis.

Solution:

stratum corneum

Exercise:

Problem:

The mucous membrane that covers the surface of the eyeball and inner eyelid is called the _____.

Solution:

conjunctiva

Short Answer

Exercise:

Problem: What is the role of keratin in the skin?

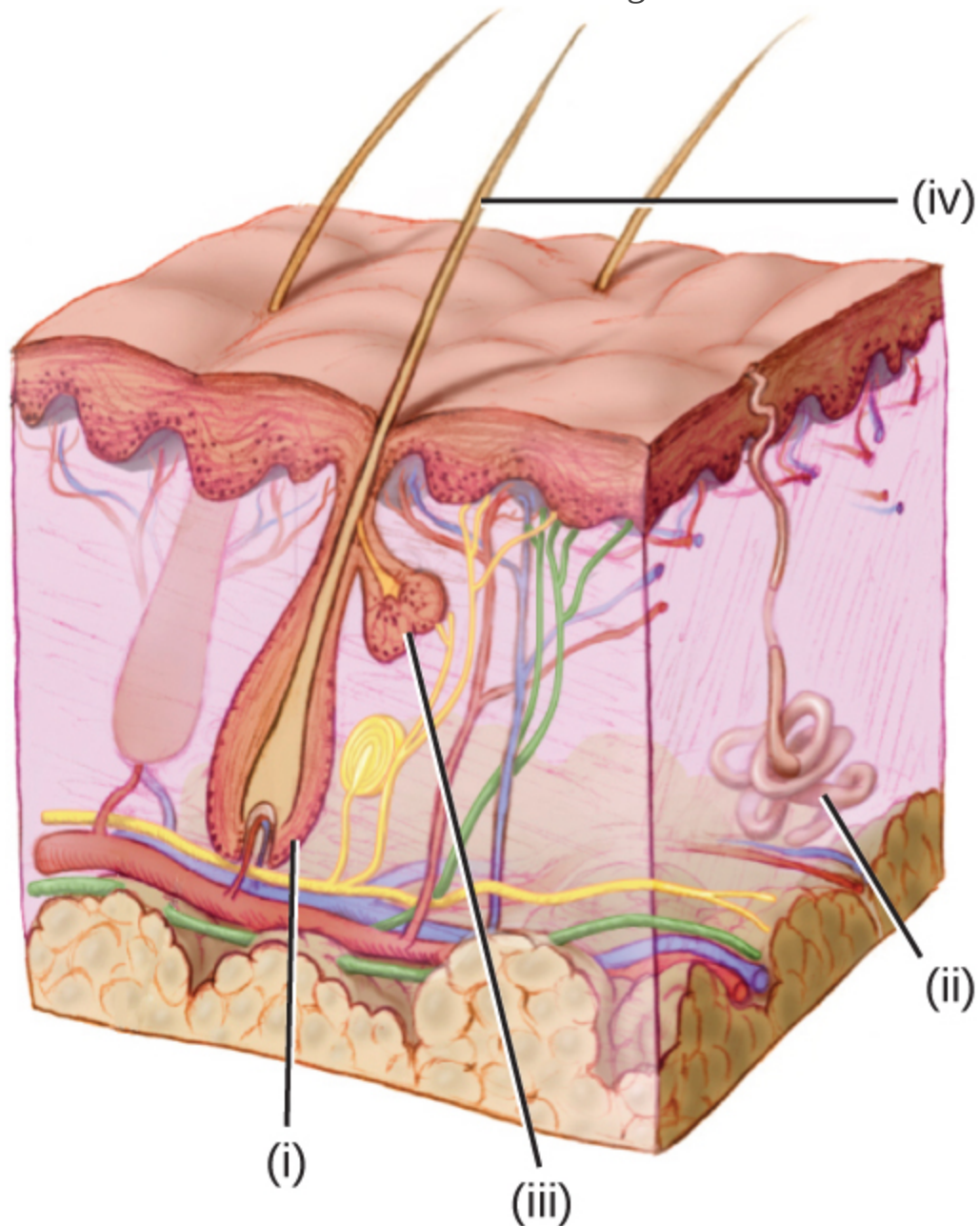
Exercise:

Problem:

What are two ways in which tears help to prevent microbial colonization?

Exercise:

Problem: Which label indicates a sweat gland?



(credit: modification of work by National Cancer Institute)

Critical Thinking

Exercise:

Problem:

Explain why it is important to understand the normal microbiota of the skin.

Exercise:

Problem:

Besides the presence or absence of ulceration, how do acute ulcerative and nonulcerative blepharitis differ?

Bacterial Infections of the Skin and Eyes

LEARNING OBJECTIVES

- Identify the most common bacterial pathogens that cause infections of the skin and eyes
- Compare the major characteristics of specific bacterial diseases affecting the skin and eyes

Despite the skin's protective functions, infections are common. Gram-positive *Staphylococcus* spp. and *Streptococcus* spp. are responsible for many of the most common skin infections. However, many skin conditions are not strictly associated with a single pathogen. Opportunistic pathogens of many types may infect skin wounds, and individual cases with identical symptoms may result from different pathogens or combinations of pathogens.

In this section, we will examine some of the most important bacterial infections of the skin and eyes and discuss how biofilms can contribute to and exacerbate such infections. Key features of bacterial skin and eye infections are also summarized in the Disease Profile boxes throughout this section.

Staphylococcal Infections of the Skin

Staphylococcus species are commonly found on the skin, with *S. epidermidis* and *S. hominis* being prevalent in the normal microbiota. *S. aureus* is also commonly found in the nasal passages and on healthy skin,

but pathogenic strains are often the cause of a broad range of infections of the skin and other body systems.

S. aureus is quite contagious. It is spread easily through skin-to-skin contact, and because many people are chronic nasal carriers (asymptomatic individuals who carry *S. aureus* in their nares), the bacteria can easily be transferred from the nose to the hands and then to fomites or other individuals. Because it is so contagious, *S. aureus* is prevalent in most community settings. This prevalence is particularly problematic in hospitals, where antibiotic-resistant strains of the bacteria may be present, and where immunocompromised patients may be more susceptible to infection. Resistant strains include methicillin-resistant *S. aureus* (MRSA), which can be acquired through health-care settings (hospital-acquired MRSA, or HA-MRSA) or in the community (community-acquired MRSA, or CA-MRSA). Hospital patients often arrive at health-care facilities already colonized with antibiotic-resistant strains of *S. aureus* that can be transferred to health-care providers and other patients. Some hospitals have attempted to detect these individuals in order to institute prophylactic measures, but they have had mixed success (see [Eye on Ethics: Screening Patients for MRSA](#)).

When a staphylococcal infection develops, choice of medication is important. As discussed above, many staphylococci (such as MRSA) are resistant to some or many antibiotics. Thus, antibiotic sensitivity is measured to identify the most suitable antibiotic. However, even before receiving the results of sensitivity analysis, suspected *S. aureus* infections are often initially treated with drugs known to be effective against MRSA, such as trimethoprim-sulfamethoxazole (TMP/SMZ), clindamycin, a tetracycline (doxycycline or minocycline), or linezolid.

The pathogenicity of staphylococcal infections is often enhanced by characteristic chemicals secreted by some strains. Staphylococcal virulence factors include hemolysins called **staphylolysins**, which are cytotoxic for many types of cells, including skin cells and white blood cells. Virulent strains of *S. aureus* are also coagulase-positive, meaning they produce coagulase, a plasma-clotting protein that is involved in abscess formation. They may also produce leukocidins, which kill white blood cells and can

contribute to the production of pus and Protein A, which inhibits phagocytosis by binding to the constant region of antibodies. Some virulent strains of *S. aureus* also produce other toxins, such as toxic shock syndrome toxin-1 (see [Virulence Factors of Bacterial and Viral Pathogens](#)).

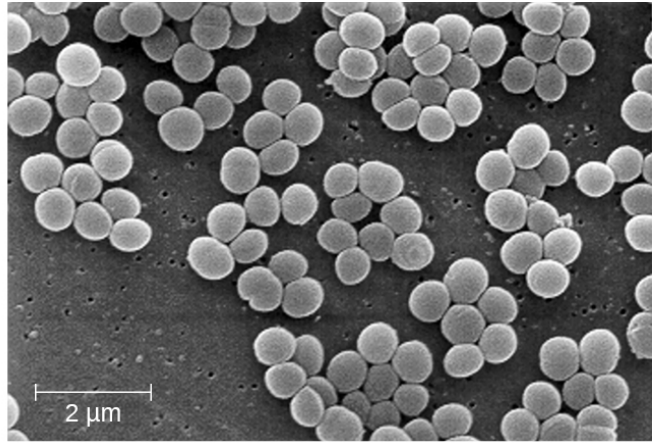
To confirm the causative agent of a suspected staphylococcal skin infection, samples from the wound are cultured. Under the microscope, gram-positive *Staphylococcus* species have cellular arrangements that form grapelike clusters; when grown on blood agar, colonies have a unique pigmentation ranging from opaque white to cream. A catalase test is used to distinguish *Staphylococcus* from *Streptococcus*, which is also a genus of gram-positive cocci and a common cause of skin infections. *Staphylococcus* species are catalase-positive while *Streptococcus* species are catalase-negative.

Other tests are performed on samples from the wound in order to distinguish coagulase-positive species of *Staphylococcus* (CoPS) such as *S. aureus* from common coagulase-negative species (CoNS) such as *S. epidermidis*. Although CoNS are less likely than CoPS to cause human disease, they can cause infections when they enter the body, as can sometimes occur via catheters, indwelling medical devices, and wounds. Passive agglutination testing can be used to distinguish CoPS from CoNS. If the sample is coagulase-positive, the sample is generally presumed to contain *S. aureus*. Additional genetic testing would be necessary to identify the particular strain of *S. aureus*.

Another way to distinguish CoPS from CoNS is by culturing the sample on mannitol salt agar (MSA). *Staphylococcus* species readily grow on this medium because they are tolerant of the high concentration of sodium chloride (7.5% NaCl). However, CoPS such as *S. aureus* ferment mannitol (which will be evident on a MSA plate), whereas CoNS such as *S. epidermidis* do not ferment mannitol but can be distinguished by the fermentation of other sugars such as lactose, malonate, and raffinose ([link](#)).



(a)



(b)

(a) A mannitol salt agar plate is used to distinguish different species of staphylococci. In this plate, *S. aureus* is on the left and *S. epidermidis* is in the right. Because *S. aureus* is capable of fermenting mannitol, it produces acids that cause the color to change to yellow. (b) This scanning electron micrograph shows the characteristic grapelike clusters of *S. aureus*. (credit a: modification of work by “ScienceProfOnline”/YouTube; credit b: modification of work by Centers for Disease Control and Prevention)

Note:

Screening Patients for MRSA

According to the CDC, 86% of invasive MRSA infections are associated in some way with healthcare, as opposed to being community-acquired. In hospitals and clinics, asymptomatic patients who harbor MRSA may spread the bacteria to individuals who are more susceptible to serious illness.

In an attempt to control the spread of MRSA, hospitals have tried screening patients for MRSA. If patients test positive following a nasal swab test, they can undergo decolonization using chlorhexidine washes or intranasal mupirocin. Some studies have reported substantial reductions in MRSA disease following implementation of these protocols, while others

have not. This is partly because there is no standard protocol for these procedures. Several different MRSA identification tests may be used, some involving slower culturing techniques and others rapid testing. Other factors, such as the effectiveness of general hand-washing protocols, may also play a role in helping to prevent MRSA transmission. There are still other questions that need to be addressed: How frequently should patients be screened? Which individuals should be tested? From where on the body should samples be collected? Will increased resistance develop from the decolonization procedures?

Even if identification and decolonization procedures are perfected, ethical questions will remain. Should patients have the right to decline testing? Should a patient who tests positive for MRSA have the right to decline the decolonization procedure, and if so, should hospitals have the right to refuse treatment to the patient? How do we balance the individual's right to receive care with the rights of other patients who could be exposed to disease as a result?

Superficial Staphylococcal Infections

S. aureus is often associated with **pyoderma**, skin infections that are **purulent**. Pus formation occurs because many strains of *S. aureus* produce leukocidins, which kill white blood cells. These purulent skin infections may initially manifest as **folliculitis**, but can lead to **furuncles** or deeper abscesses called **carbuncles**.

Folliculitis generally presents as bumps and pimples that may be itchy, red, and/or pus-filled. In some cases, folliculitis is self-limiting, but if it continues for more than a few days, worsens, or returns repeatedly, it may require medical treatment. Sweat, skin injuries, ingrown hairs, tight clothing, irritation from shaving, and skin conditions can all contribute to folliculitis. Avoidance of tight clothing and skin irritation can help to prevent infection, but topical antibiotics (and sometimes other treatments) may also help. Folliculitis can be identified by skin inspection; treatment is generally started without first culturing and identifying the causative agent.

In contrast, furuncles (boils) are deeper infections ([\[link\]](#)). They are most common in those individuals (especially young adults and teenagers) who play contact sports, share athletic equipment, have poor nutrition, live in close quarters, or have weakened immune systems. Good hygiene and skin care can often help to prevent furuncles from becoming more infective, and they generally resolve on their own. However, if furuncles spread, increase in number or size, or lead to systemic symptoms such as fever and chills, then medical care is needed. They may sometimes need to be drained (at which time the pathogens can be cultured) and treated with antibiotics.

When multiple boils develop into a deeper lesion, it is called a carbuncle ([\[link\]](#)). Because carbuncles are deeper, they are more commonly associated with systemic symptoms and a general feeling of illness. Larger, recurrent, or worsening carbuncles require medical treatment, as do those associated with signs of illness such as fever. Carbuncles generally need to be drained and treated with antibiotics. While carbuncles are relatively easy to identify visually, culturing and laboratory analysis of the wound may be recommended for some infections because antibiotic resistance is relatively common.

Proper hygiene is important to prevent these types of skin infections or to prevent the progression of existing infections.



(a)



(b)

Furuncles (boils) and carbuncles are infections of the skin often caused

by *Staphylococcus* bacteria. (a) A furuncle contains pus and exhibits swelling. (b) A carbuncle is a pus-filled lesion that is typically deeper than the furuncle. It often forms from multiple furuncles. (credit a: modification of work by “Mahdouch”/Wikimedia Commons; credit b: modification of work by “Drvgaikwad”/Wikimedia Commons)

Staphylococcal scalded skin syndrome (SSSS) is another superficial infection caused by *S. aureus* that is most commonly seen in young children, especially infants. Bacterial exotoxins first produce **erythema** (redness of the skin) and then severe peeling of the skin, as might occur after scalding ([link](#)). SSSS is diagnosed by examining characteristics of the skin (which may rub off easily), using blood tests to check for elevated white blood cell counts, culturing, and other methods. Intravenous antibiotics and fluid therapy are used as treatment.



A newborn with staphylococcal scalded skin syndrome (SSSS), which results in large regions of peeling, dead

skin. (credit: modification of work by D Jeyakumari, R Gopal, M Eswaran, and C MaheshKumar)

Impetigo

The skin infection **impetigo** causes the formation of vesicles, pustules, and possibly bullae, often around the nose and mouth. Bullae are large, fluid-filled blisters that measure at least 5 mm in diameter. Impetigo can be diagnosed as either nonbullous or bullous. In nonbullous impetigo, vesicles and pustules rupture and become encrusted sores. Typically the crust is yellowish, often with exudate draining from the base of the lesion. In bullous impetigo, the bullae fill and rupture, resulting in larger, draining, encrusted lesions ([\[link\]](#)).

Especially common in children, impetigo is particularly concerning because it is highly contagious. Impetigo can be caused by *S. aureus* alone, by *Streptococcus pyogenes* alone, or by coinfection of *S. aureus* and *S. pyogenes*. Impetigo is often diagnosed through observation of its characteristic appearance, although culture and susceptibility testing may also be used.

Topical or oral antibiotic treatment is typically effective in treating most cases of impetigo. However, cases caused by *S. pyogenes* can lead to serious sequelae (pathological conditions resulting from infection, disease, injury, therapy, or other trauma) such as acute glomerulonephritis (AGN), which is severe inflammation in the kidneys.



Impetigo is characterized by vesicles, pustules, or bullae that rupture, producing encrusted sores. (credit: modification of work by FDA)

Nosocomial *S. epidermidis* Infections

Though not as virulent as *S. aureus*, the staphylococcus *S. epidermidis* can cause serious opportunistic infections. Such infections usually occur only in hospital settings. *S. epidermidis* is usually a harmless resident of the normal skin microbiota. However, health-care workers can inadvertently transfer *S. epidermidis* to medical devices that are inserted into the body, such as catheters, prostheses, and indwelling medical devices. Once it has bypassed the skin barrier, *S. epidermidis* can cause infections inside the body that can

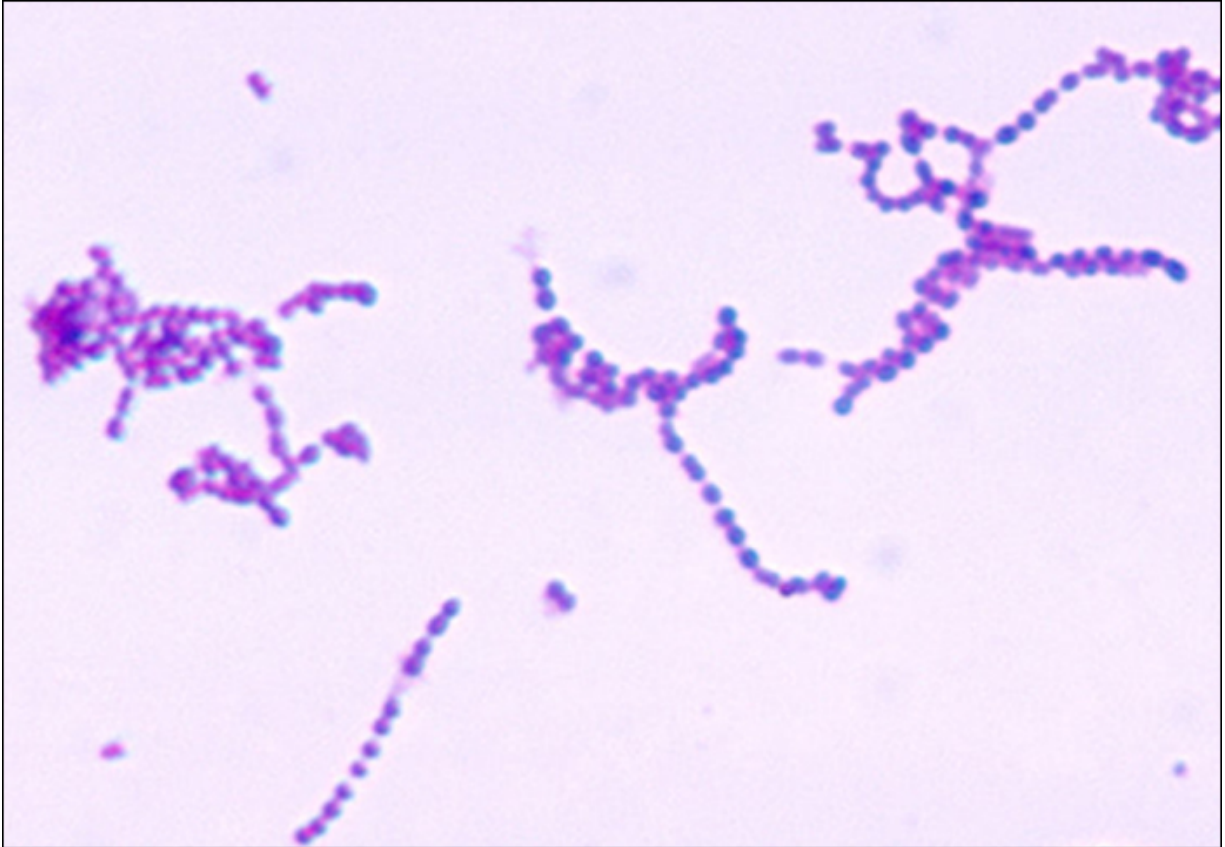
be difficult to treat. Like *S. aureus*, *S. epidermidis* is resistant to many antibiotics, and localized infections can become systemic if not treated quickly. To reduce the risk of nosocomial (hospital-acquired) *S. epidermidis*, health-care workers must follow strict procedures for handling and sterilizing medical devices before and during surgical procedures.

Note:

- Why are *Staphylococcus aureus* infections often purulent?

Streptococcal Infections of the Skin

Streptococcus are gram-positive cocci with a microscopic morphology that resembles chains of bacteria. Colonies are typically small (1–2 mm in diameter), translucent, entire edge, with a slightly raised elevation that can be either nonhemolytic, alpha-hemolytic, or beta-hemolytic when grown on blood agar ([\[link\]](#)). Additionally, they are facultative anaerobes that are catalase-negative.



Streptococcus pyogenes forms chains of cocci. (credit: modification of work by Centers for Disease Control and Prevention)

The genus *Streptococcus* includes important pathogens that are categorized in serological Lancefield groups based on the distinguishing characteristics of their surface carbohydrates. The most clinically important streptococcal species in humans is *S. pyogenes*, also known as group A streptococcus (GAS). *S. pyogenes* produces a variety of extracellular enzymes, including streptolysins O and S, hyaluronidase, and streptokinase. These enzymes can aid in transmission and contribute to the inflammatory response.[\[footnote\]](#) *S. pyogenes* also produces a capsule and **M protein**, a streptococcal cell wall protein. These virulence factors help the bacteria to avoid phagocytosis while provoking a substantial immune response that contributes to symptoms associated with streptococcal infections.

Starr, C.R. and Engelberg N.C. "Role of Hyaluronidase in Subcutaneous Spread and Growth of Group A Streptococcus." *Infection and Immunity*

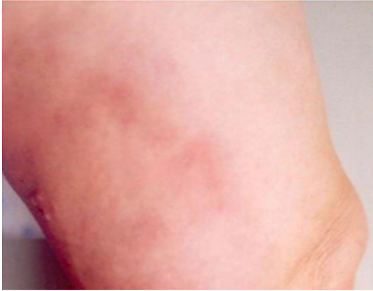
2006(7:1): 40–48. doi: 10.1128/IAI.74.1.40-48.2006.

S. pyogenes causes a wide variety of diseases not only in the skin, but in other organ systems as well. Examples of diseases elsewhere in the body include pharyngitis and scarlet fever, which will be covered in later chapters.

Cellulitis, Erysipelas, and Erythema Nodosum

Common streptococcal conditions of the skin include cellulitis, erysipelas, and erythema nodosum. An infection that develops in the dermis or hypodermis can cause **cellulitis**, which presents as a reddened area of the skin that is warm to the touch and painful. The causative agent is often *S. pyogenes*, which may breach the epidermis through a cut or abrasion, although cellulitis may also be caused by staphylococci. *S. pyogenes* can also cause **erysipelas**, a condition that presents as a large, intensely inflamed patch of skin involving the dermis (often on the legs or face). These infections can be **suppurative**, which results in a bullous form of erysipelas. Streptococcal and other pathogens may also cause a condition called **erythema nodosum**, characterized by inflammation in the subcutaneous fat cells of the hypodermis. It sometimes results from a streptococcal infection, though other pathogens can also cause the condition. It is not suppurative, but leads to red nodules on the skin, most frequently on the shins ([\[link\]](#)).

In general, streptococcal infections are best treated through identification of the specific pathogen followed by treatment based upon that particular pathogen's susceptibility to different antibiotics. Many immunological tests, including agglutination reactions and ELISAs, can be used to detect streptococci. Penicillin is commonly prescribed for treatment of cellulitis and erysipelas because resistance is not widespread in streptococci at this time. In most patients, erythema nodosum is self-limiting and is not treated with antimicrobial drugs. Recommended treatments may include nonsteroidal anti-inflammatory drugs (NSAIDs), cool wet compresses, elevation, and bed rest.



(a)



(b)



(c)

S. pyogenes can cause a variety of skin conditions once it breaches the skin barrier through a cut or wound. (a) Cellulitis presents as a painful, red rash. (b) Erysipelas presents as a raised rash, usually with clear borders. (c) Erythema nodosum is characterized by red lumps or nodules, typically on the lower legs. (credit a: modification of work by “Bassukas ID, Gaitanis G, Zioga A, Boboyianni C, Stergiopoulou C; credit b: modification of work by Centers for Disease Control and Prevention; credit c: modification of work by Dean C, Crow WT)

Necrotizing Fasciitis

Streptococcal infections that start in the skin can sometimes spread elsewhere, resulting in a rare but potentially life-threatening condition called **necrotizing fasciitis**, sometimes referred to as flesh-eating bacterial syndrome. *S. pyogenes* is one of several species that can cause this rare but potentially-fatal condition; others include *Klebsiella*, *Clostridium*, *Escherichia coli*, *S. aureus*, and *Aeromonas hydrophila*.

Necrotizing fasciitis occurs when the fascia, a thin layer of connective tissue between the skin and muscle, becomes infected. Severe invasive necrotizing fasciitis due to *Streptococcus pyogenes* occurs when virulence factors that are responsible for adhesion and invasion overcome host defenses. *S. pyogenes* invasins allow bacterial cells to adhere to tissues and establish infection. Bacterial proteases unique to *S. pyogenes* aggressively infiltrate and destroy host tissues, inactivate complement, and prevent

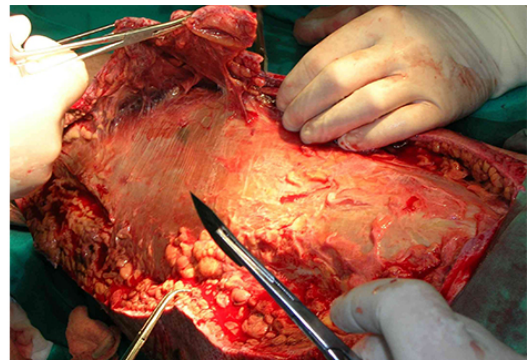
neutrophil migration to the site of infection. The infection and resulting tissue death can spread very rapidly, as large areas of skin become detached and die. Treatment generally requires debridement (surgical removal of dead or infected tissue) or amputation of infected limbs to stop the spread of the infection; surgical treatment is supplemented with intravenous antibiotics and other therapies ([\[link\]](#)).

Necrotizing fasciitis does not always originate from a skin infection; in some cases there is no known portal of entry. Some studies have suggested that experiencing a blunt force trauma can increase the risk of developing streptococcal necrotizing fasciitis.[\[footnote\]](#)

Nuwayhid, Z.B., Aronoff, D.M., and Mulla, Z.D.. “Blunt Trauma as a Risk Factor for Group A Streptococcal Necrotizing Fasciitis.” *Annals of Epidemiology* (2007) 17:878–881.



(a)



(b)

(a) The left leg of this patient shows the clinical features of necrotizing fasciitis. (b) The same patient's leg is surgically debrided to remove the infection. (credit a, b: modification of work by Piotr Smuszkiewicz, Iwona Trojanowska, and Hanna Tomczak)

Note:

- How do staphylococcal infections differ in general presentation from streptococcal infections?

Note:

Part 2

Observing that Sam's wound is purulent, the doctor tells him that he probably has a bacterial infection. She takes a sample from the lesion to send for laboratory analysis, but because it is Friday, she does not expect to receive the results until the following Monday. In the meantime, she prescribes an over-the-counter topical antibiotic ointment. She tells Sam to keep the wound clean and apply a new bandage with the ointment at least twice per day.

- How would the lab technician determine if the infection is staphylococcal or streptococcal? Suggest several specific methods.
- What tests might the lab perform to determine the best course of antibiotic treatment?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

***Pseudomonas* Infections of the Skin**

Another important skin pathogen is *Pseudomonas aeruginosa*, a gram-negative, oxidase-positive, aerobic bacillus that is commonly found in water and soil as well as on human skin. *P. aeruginosa* is a common cause of opportunistic infections of wounds and burns. It can also cause hot tub rash, a condition characterized by folliculitis that frequently afflicts users of pools and hot tubs (recall the Clinical Focus case in [Microbial Biochemistry](#)). *P. aeruginosa* is also the cause of **otitis externa** (swimmer's ear), an infection of the ear canal that causes itching, redness, and discomfort, and can progress to fever, pain, and swelling ([link](#)).



(a)

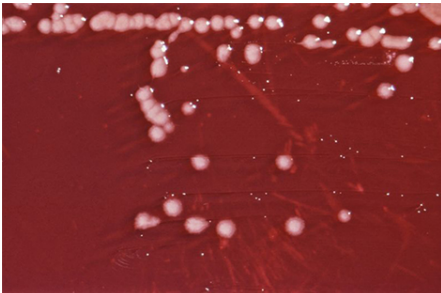


(b)

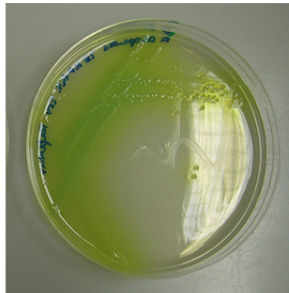
(a) Hot tub folliculitis presents as an itchy red rash. It is typically caused by *P. aeruginosa*, a bacterium that thrives in wet, warm environments such as hot tubs. (b) Otitis externa (swimmer's ear) may also be caused by *P. aeruginosa* or other bacteria commonly found in water. Inflammation of the outer ear and ear canal can lead to painful swelling. (credit b: modification of work by Klaus D. Peter)

Wounds infected with *P. aeruginosa* have a distinctive odor resembling grape soda or fresh corn tortillas. This odor is caused by the 2-aminoacetophenone that is used by *P. aeruginosa* in quorum sensing and contributes to its pathogenicity. Wounds infected with certain strains of *P. aeruginosa* also produce a blue-green pus due to the pigments **pyocyanin** and **pyoverdinin**, which also contribute to its virulence. Pyocyanin and pyoverdinin are siderophores that help *P. aeruginosa* survive in low-iron environments by enhancing iron uptake. *P. aeruginosa* also produces several other virulence factors, including phospholipase C (a hemolysin capable of breaking down red blood cells), exoenzyme S (involved in adherence to epithelial cells), and exotoxin A (capable of causing tissue necrosis). Other virulence factors include a slime that allows the bacterium to avoid being phagocytized, fimbriae for adherence, and proteases that

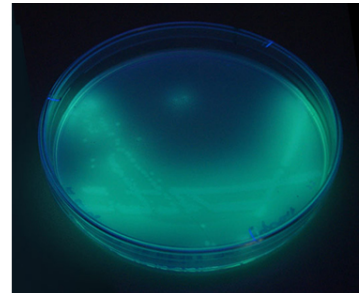
cause tissue damage. *P. aeruginosa* can be detected through the use of cetrimide agar, which is selective for *Pseudomonas* species ([\[link\]](#)).



(a)



(b)



(c)

(a) These *P. aeruginosa* colonies are growing on xylose lysine sodium deoxycholate (XLD) agar. (b) *Pseudomonas* spp. can produce a variety of blue-green pigments. (c) *Pseudomonas* spp. may produce fluorescein, which fluoresces green under ultraviolet light under the right conditions. (credit a: modification of work by Centers for Disease Control and Prevention)

Pseudomonas spp. tend to be resistant to most antibiotics. They often produce β -lactamases, may have mutations affecting porins (small cell wall channels) that affect antibiotic uptake, and may pump some antibiotics out of the cell, contributing to this resistance. Polymyxin B and gentamicin are effective, as are some fluoroquinolones. Otitis externa is typically treated with ear drops containing acetic acid, antibacterials, and/or steroids to reduce inflammation; ear drops may also include antifungals because fungi can sometimes cause or contribute to otitis externa. Wound infections caused by *Pseudomonas* spp. may be treated with topical antibiofilm agents that disrupt the formation of biofilms.

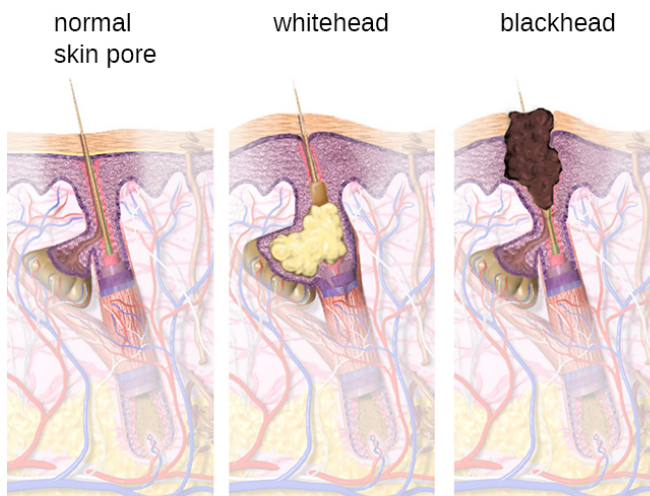
Note:

- Name at least two types of skin infections commonly caused by *Pseudomonas* spp.

Acne

One of the most ubiquitous skin conditions is **acne**. Acne afflicts nearly 80% of teenagers and young adults, but it can be found in individuals of all ages. Higher incidence among adolescents is due to hormonal changes that can result in overproduction of sebum.

Acne occurs when hair follicles become clogged by shed skin cells and sebum, causing non-inflammatory lesions called comedones. Comedones (singular “comedo”) can take the form of whitehead and blackhead pimples. Whiteheads are covered by skin, whereas blackhead pimples are not; the black color occurs when lipids in the clogged follicle become exposed to the air and oxidize ([link](#)).



(a)



(b)

(a) Acne is characterized by whitehead and blackhead comedones that result from clogged hair follicles. (b) Blackheads, visible as black spots on the skin, have a dark appearance due to the oxidation of lipids

in sebum via exposure to the air. (credit a: modification of work by Bruce Blaus)

Often comedones lead to infection by *Propionibacterium acnes*, a gram-positive, non-spore-forming, aerotolerant anaerobic bacillus found on skin that consumes components of sebum. *P. acnes* secretes enzymes that damage the hair follicle, causing inflammatory lesions that may include papules, pustules, nodules, or pseudocysts, depending on their size and severity.

Treatment of acne depends on the severity of the case. There are multiple ways to grade acne severity, but three levels are usually considered based on the number of comedones, the number of inflammatory lesions, and the types of lesions. Mild acne is treated with topical agents that may include salicylic acid (which helps to remove old skin cells) or retinoids (which have multiple mechanisms, including the reduction of inflammation). Moderate acne may be treated with antibiotics (erythromycin, clindamycin), acne creams (e.g., benzoyl peroxide), and hormones. Severe acne may require treatment using strong medications such as isotretinoin (a retinoid that reduces oil buildup, among other effects, but that also has serious side effects such as photosensitivity). Other treatments, such as phototherapy and laser therapy to kill bacteria and possibly reduce oil production, are also sometimes used.

Note:

- What is the role of *Propionibacterium acnes* in causing acne?

Note:

Resolution

Sam uses the topical antibiotic over the weekend to treat his wound, but he does not see any improvement. On Monday, the doctor calls to inform him that the results from his laboratory tests are in. The tests show evidence of both *Staphylococcus* and *Streptococcus* in his wound. The bacterial species were confirmed using several tests. A passive agglutination test confirmed the presence of *S. aureus*. In this type of test, latex beads with antibodies cause agglutination when *S. aureus* is present. *Streptococcus pyogenes* was confirmed in the wound based on bacitracin (0.04 units) susceptibility as well as latex agglutination tests specific for *S. pyogenes*.

Because many strains of *S. aureus* are resistant to antibiotics, the doctor had also requested an antimicrobial susceptibility test (AST) at the same time the specimen was submitted for identification. The results of the AST indicated no drug resistance for the *Streptococcus* spp.; the *Staphylococcus* spp. showed resistance to several common antibiotics, but were susceptible to cefoxitin and oxacillin. Once Sam began to use these new antibiotics, the infection resolved within a week and the lesion healed.

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Anthrax

The zoonotic disease **anthrax** is caused by *Bacillus anthracis*, a gram-positive, endospore-forming, facultative anaerobe. Anthrax mainly affects animals such as sheep, goats, cattle, and deer, but can be found in humans as well. Sometimes called wool sorter's disease, it is often transmitted to humans through contact with infected animals or animal products, such as wool or hides. However, exposure to *B. anthracis* can occur by other means, as the endospores are widespread in soils and can survive for long periods of time, sometimes for hundreds of years.

The vast majority of anthrax cases (95–99%) occur when anthrax endospores enter the body through abrasions of the skin.[\[footnote\]](#) This form of the disease is called cutaneous anthrax. It is characterized by the formation of a nodule on the skin; the cells within the nodule die, forming a black **eschar**, a mass of dead skin tissue ([\[link\]](#)). The localized infection can eventually lead to bacteremia and septicemia. If untreated, cutaneous

anthrax can cause death in 20% of patients.[\[footnote\]](#) Once in the skin tissues, *B. anthracis* endospores germinate and produce a capsule, which prevents the bacteria from being phagocytized, and two binary exotoxins that cause edema and tissue damage. The first of the two exotoxins consists of a combination of protective antigen (PA) and an enzymatic lethal factor (LF), forming lethal toxin (LeTX). The second consists of protective antigen (PA) and an edema factor (EF), forming edema toxin (EdTX). Shadomy, S.V., Traxler, R.M., and Marston, C.K. “Infectious Diseases Related to Travel: Anthrax” 2015. Centers for Disease Control and Prevention. <http://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/anthrax>. Accessed Sept 14, 2016.

US FDA. “Anthrax.” 2015. <http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ucm061751.htm>. Accessed Sept 14, 2016.



(a)



(b)

(a) Cutaneous anthrax is an infection of the skin by *B. anthracis*, which produces tissue-damaging exotoxins. Dead tissues accumulating in this nodule have produced a small black eschar. (b) Colonies of *B. anthracis* grown on sheep's blood agar. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Less commonly, anthrax infections can be initiated through other portals of entry such as the digestive tract (gastrointestinal anthrax) or respiratory tract (pulmonary anthrax or inhalation anthrax). Typically, cases of noncutaneous anthrax are more difficult to treat than the cutaneous form. The mortality rate for gastrointestinal anthrax can be up to 40%, even with treatment. Inhalation anthrax, which occurs when anthrax spores are inhaled, initially causes influenza-like symptoms, but mortality rates are approximately 45% in treated individuals and 85% in those not treated. A relatively new form of the disease, injection anthrax, has been reported in Europe in intravenous drug users; it occurs when drugs are contaminated with *B. anthracis*. Patients with injection anthrax show signs and symptoms of severe soft tissue infection that differ clinically from cutaneous anthrax. This often delays diagnosis and treatment, and leads to a high mortality rate.[\[footnote\]](#)

Berger, T., Kassirer, M., and Aran, A.A.. “Injectional Anthrax—New Presentation of an Old Disease.” *Euro Surveill* 19 (2014) 32.
<http://www.ncbi.nlm.nih.gov/pubmed/25139073>. Accessed Sept 14, 2016.

B. anthracis colonies on blood agar have a rough texture and serrated edges that eventually form an undulating band ([\[link\]](#)). Broad spectrum antibiotics such as penicillin, erythromycin, and tetracycline are often effective treatments.

Unfortunately, *B. anthracis* has been used as a biological weapon and remains on the United Nations’ list of potential agents of bioterrorism. [\[footnote\]](#) Over a period of several months in 2001, a number of letters were mailed to members of the news media and the United States Congress. As a result, 11 individuals developed cutaneous anthrax and another 11 developed inhalation anthrax. Those infected included recipients of the letters, postal workers, and two other individuals. Five of those infected with pulmonary anthrax died. The anthrax spores had been carefully prepared to aerosolize, showing that the perpetrator had a high level of expertise in microbiology.[\[footnote\]](#)

United Nations Office at Geneva. “What Are Biological and Toxin Weapons?”
<http://www.unog.ch/80256EE600585943/%28httpPages%29/29B727532FECBE96C12571860035A6DB?>. Accessed Sept 14, 2016.

Federal Bureau of Investigation. “Famous Cases and Criminals: Amerithrax or Anthrax Investigation.” <https://www.fbi.gov/history/famous-cases/amerithrax-or-anthrax-investigation>. Accessed Sept 14, 2016.

A vaccine is available to protect individuals from anthrax. However, unlike most routine vaccines, the current anthrax vaccine is unique in both its formulation and the protocols dictating who receives it.^[footnote] The vaccine is administered through five intramuscular injections over a period of 18 months, followed by annual boosters. The US Food and Drug Administration (FDA) has only approved administration of the vaccine prior to exposure for at-risk adults, such as individuals who work with anthrax in a laboratory, some individuals who handle animals or animal products (e.g., some veterinarians), and some members of the United States military. The vaccine protects against cutaneous and inhalation anthrax using cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *B. anthracis*.^[footnote] The FDA has not approved the vaccine for routine use *after* exposure to anthrax, but if there were ever an anthrax emergency in the United States, patients could be given anthrax vaccine after exposure to help prevent disease.

Centers for Disease Control and Prevention. “Anthrax: Medical Care: Prevention: Antibiotics.” <http://www.cdc.gov/anthrax/medical-care/prevention.html>. Accessed Sept 14, 2016.

Emergent Biosolutions. AVA (BioThrax) vaccine package insert (Draft). Nov 2015.

<http://www.fda.gov/downloads/biologicsbloodvaccines/bloodbloodproducts/approvedproducts/licensedproductsblas/ucm074923.pdf>.

Note:

- What is the characteristic feature of a cutaneous anthrax infection?

Note:

Bacterial Infections of the Skin

Bacterial infections of the skin can cause a wide range of symptoms and syndromes, ranging from the superficial and relatively harmless to the severe and even fatal. Most bacterial skin infections can be diagnosed by culturing the bacteria and treated with antibiotics. Antimicrobial susceptibility testing is also often necessary because many strains of bacteria have developed antibiotic resistance. [\[link\]](#) summarizes the characteristics of some common bacterial skin infections.

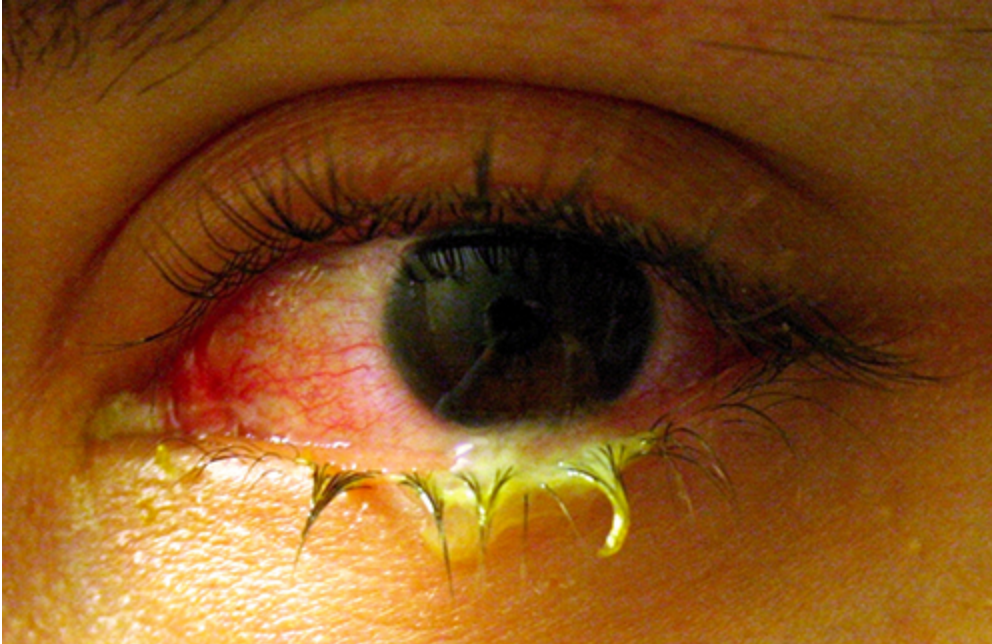
Bacterial Infections of the Skin				
Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Acne	<i>Propionibacterium acnes</i>	Comedones (whiteheads, blackheads); papules, pustules, nodules, or pseudocysts	Not transmissible; clogged pores become infected by normal skin microbiota (<i>P. acnes</i>)	Erythromycin, clindamycin
Anthrax (cutaneous)	<i>Bacillus anthracis</i>	Eschar at site of infection; may lead to septicemia and can be fatal	Entry of <i>B. anthracis</i> endospores through cut or abrasion	Penicillin, erythromycin, or tetracycline
Cellulitis	<i>Streptococcus pyogenes</i>	Localized inflammation of dermis and hypodermis; skin red, warm, and painful to the touch	Entry of <i>S. pyogenes</i> through cut or abrasion	Oral or intravenous antibiotics (e.g., penicillin)
Erysipelas	<i>S. pyogenes</i>	Inflamed, swollen patch of skin, often on face; may be suppurative	Entry of <i>S. pyogenes</i> through cut or abrasion	Oral or intravenous antibiotics (e.g., penicillin)
Erythema nodosum	<i>S. pyogenes</i>	Small red nodules, often on shins	Associated with other streptococcal infection	None or anti-inflammatory drugs for severe cases
Impetigo	<i>Staphylococcus aureus</i> , <i>S. pyogenes</i>	Vesicles, pustules, and sometimes bullae around nose and mouth	Highly contagious, especially via contact	Topical or oral antibiotics
Necrotizing fasciitis	<i>S. pyogenes</i> , <i>Klebsiella</i> , <i>Clostridium</i> , others	Infection of fascia and rapidly spreading tissue death; can lead to septic shock and death	Entry of bacteria through cut or abrasion	Intravenous broad-spectrum antibiotics
Otitis externa	<i>Pseudomonas aeruginosa</i>	Itching, redness, discomfort of ear canal, progressing to fever, pain, swelling	<i>P. aeruginosa</i> enters ear canal via pool or other water	Acidic ear drops with antibiotics, antifungals, steroids
Staphylococcal scalded skin syndrome (SSSS)	<i>S. aureus</i>	Erythema and severe peeling of skin	Infection of skin and mucous membranes, especially in children	Intravenous antibiotics, fluid therapy
Wound infections	<i>P. aeruginosa</i> , others	Formation of biofilm in or on wound	Exposure of wound to microbes in environment; poor wound hygiene	Polymyxin B, gentamicin, fluoroquinolones, topical anti-biofilm agents

Bacterial Conjunctivitis

Like the skin, the surface of the eye comes in contact with the outside world and is somewhat prone to infection by bacteria in the environment.

Bacterial conjunctivitis (pinkeye) is a condition characterized by inflammation of the conjunctiva, often accompanied by a discharge of sticky fluid (described as acute purulent conjunctivitis) ([\[link\]](#)).

Conjunctivitis can affect one eye or both, and it usually does not affect vision permanently. Bacterial conjunctivitis is most commonly caused by *Haemophilus influenzae*, but can also be caused by other species such as *Moraxella catarrhalis*, *S. pneumoniae*, and *S. aureus*. The causative agent may be identified using bacterial cultures, Gram stain, and diagnostic biochemical, antigenic, or nucleic acid profile tests of the isolated pathogen. Bacterial conjunctivitis is very contagious, being transmitted via secretions from infected individuals, but it is also self-limiting. Bacterial conjunctivitis usually resolves in a few days, but topical antibiotics are sometimes prescribed. Because this condition is so contagious, medical attention is recommended whenever it is suspected. Individuals who use contact lenses should discontinue their use when conjunctivitis is suspected. Certain symptoms, such as blurred vision, eye pain, and light sensitivity, can be associated with serious conditions and require medical attention.



Acute, purulent, bacterial conjunctivitis causes swelling and redness in the conjunctiva, the membrane lining the whites of the eyes and the inner eyelids. It is often accompanied by a yellow, green, or white discharge, which can dry and become encrusted on the eyelashes.
(credit: “Tanalai”/Wikimedia Commons)

Neonatal Conjunctivitis

Newborns whose mothers have certain sexually transmitted infections are at risk of contracting **ophthalmia neonatorum** or **inclusion conjunctivitis**, which are two forms of neonatal conjunctivitis contracted through exposure to pathogens during passage through the birth canal. Gonococcal ophthalmia neonatorum is caused by *Neisseria gonorrhoeae*, the bacterium that causes the STD gonorrhea ([\[link\]](#)). Inclusion (chlamydial) conjunctivitis is caused by *Chlamydia trachomatis*, the anaerobic, obligate, intracellular parasite that causes the STD chlamydia.

To prevent gonococcal ophthalmia neonatorum, silver nitrate ointments were once routinely applied to all infants' eyes shortly after birth; however, it is now more common to apply antibacterial creams or drops, such as erythromycin. Most hospitals are required by law to provide this preventative treatment to all infants, because conjunctivitis caused by *N. gonorrhoeae*, *C. trachomatis*, or other bacteria acquired during a vaginal delivery can have serious complications. If untreated, the infection can spread to the cornea, resulting in ulceration or perforation that can cause vision loss or even permanent blindness. As such, neonatal conjunctivitis is treated aggressively with oral or intravenous antibiotics to stop the spread of the infection. Causative agents of inclusion conjunctivitis may be identified using bacterial cultures, Gram stain, and diagnostic biochemical, antigenic, or nucleic acid profile tests.



A newborn suffering from gonococcal ophthalmia neonatorum. Left untreated, purulent discharge can scar the cornea, causing loss of vision or permanent blindness. (credit: Centers for Disease Control and Prevention)

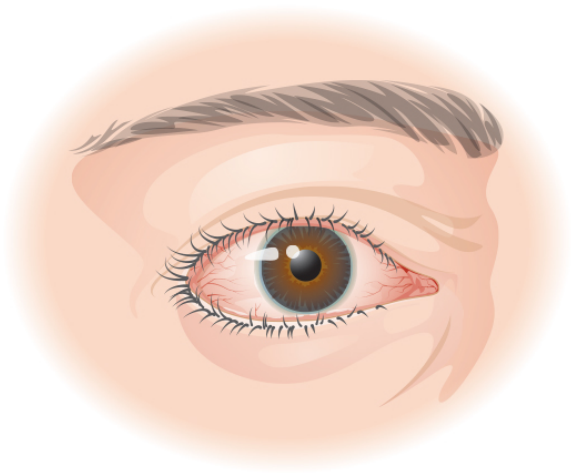
Note:

- Compare and contrast bacterial conjunctivitis with neonatal conjunctivitis.

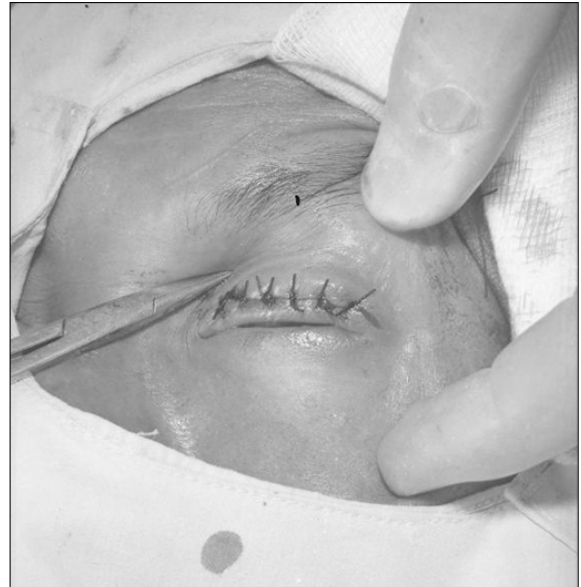
Trachoma

Trachoma, or granular conjunctivitis, is a common cause of preventable blindness that is rare in the United States but widespread in developing countries, especially in Africa and Asia. The condition is caused by the same species that causes neonatal inclusion conjunctivitis in infants, *Chlamydia trachomatis*. *C. trachomatis* can be transmitted easily through fomites such as contaminated towels, bed linens, and clothing and also by direct contact with infected individuals. *C. trachomatis* can also be spread by flies that transfer infected mucous containing *C. trachomatis* from one human to another.

Infection by *C. trachomatis* causes chronic conjunctivitis, which leads to the formation of necrotic follicles and scarring in the upper eyelid. The scars turn the eyelashes inward (a condition known as trichiasis) and mechanical abrasion of the cornea leads to blindness ([\[link\]](#)). Antibiotics such as azithromycin are effective in treating trachoma, and outcomes are good when the disease is treated promptly. In areas where this disease is common, large public health efforts are focused on reducing transmission by teaching people how to avoid the risks of the infection.



(a)



(b)

(a) If trachoma is not treated early with antibiotics, scarring on the eyelid can lead to trichiasis, a condition in which the eyelashes turn inward. (b) Trichiasis leads to blindness if not corrected by surgery, as shown here. (credit b: modification of work by Otis Historical Archives National Museum of Health & Medicine)

Note:

- Why is trachoma rare in the United States?

Note:

SAFE Eradication of Trachoma

Though uncommon in the United States and other developed nations, trachoma is the leading cause of preventable blindness worldwide, with more than 4 million people at immediate risk of blindness from trichiasis.

The vast majority of those affected by trachoma live in Africa and the Middle East in isolated rural or desert communities with limited access to clean water and sanitation. These conditions provide an environment conducive to the growth and spread of *Chlamydia trachomatis*, the bacterium that causes trachoma, via wastewater and eye-seeking flies. In response to this crisis, recent years have seen major public health efforts aimed at treating and preventing trachoma. The Alliance for Global Elimination of Trachoma by 2020 (GET 2020), coordinated by the World Health Organization (WHO), promotes an initiative dubbed “SAFE,” which stands for “Surgery, Antibiotics, Facial cleanliness, and Environmental improvement.” The Carter Center, a charitable, nongovernment organization led by former US President Jimmy Carter, has partnered with the WHO to promote the SAFE initiative in six of the most critically impacted nations in Africa. Through its Trachoma Control Program, the Carter Center trains and equips local surgeons to correct trichiasis and distributes antibiotics to treat trachoma. The program also promotes better personal hygiene through health education and improves sanitation by funding the construction of household latrines. This reduces the prevalence of open sewage, which provides breeding grounds for the flies that spread trachoma.

Bacterial Keratitis

Keratitis can have many causes, but bacterial keratitis is most frequently caused by *Staphylococcus epidermidis* and/or *Pseudomonas aeruginosa*. Contact lens users are particularly at risk for such an infection because *S. epidermidis* and *P. aeruginosa* both adhere well to the surface of the lenses. Risk of infection can be greatly reduced by proper care of contact lenses and avoiding wearing lenses overnight. Because the infection can quickly lead to blindness, prompt and aggressive treatment with antibiotics is important. The causative agent may be identified using bacterial cultures, Gram stain, and diagnostic biochemical, antigenic, or nucleic acid profile tests of the isolated pathogen.

Note:

- Why are contact lens wearers at greater risk for developing keratitis?

Biofilms and Infections of the Skin and Eyes

When treating bacterial infections of the skin and eyes, it is important to consider that few such infections can be attributed to a single pathogen. While biofilms may develop in other parts of the body, they are especially relevant to skin infections (such as those caused by *S. aureus* or *P. aeruginosa*) because of their prevalence in chronic skin wounds. Biofilms develop when bacteria (and sometimes fungi) attach to a surface and produce extracellular polymeric substances (EPS) in which cells of multiple organisms may be embedded. When a biofilm develops on a wound, it may interfere with the natural healing process as well as diagnosis and treatment.

Because biofilms vary in composition and are difficult to replicate in the lab, they are still not thoroughly understood. The extracellular matrix of a biofilm consists of polymers such as polysaccharides, extracellular DNA, proteins, and lipids, but the exact makeup varies. The organisms living within the extracellular matrix may include familiar pathogens as well as other bacteria that do not grow well in cultures (such as numerous obligate anaerobes). This presents challenges when culturing samples from infections that involve a biofilm. Because only some species grow *in vitro*, the culture may contain only a subset of the bacterial species involved in the infection.

Biofilms confer many advantages to the resident bacteria. For example, biofilms can facilitate attachment to surfaces on or in the host organism (such as wounds), inhibit phagocytosis, prevent the invasion of neutrophils, and sequester host antibodies. Additionally, biofilms can provide a level of antibiotic resistance not found in the isolated cells and colonies that are typical of laboratory cultures. The extracellular matrix provides a physical barrier to antibiotics, shielding the target cells from exposure. Moreover, cells within a biofilm may differentiate to create subpopulations of dormant

cells called persister cells. Nutrient limitations deep within a biofilm add another level of resistance, as stress responses can slow metabolism and increase drug resistance.

Note:
Bacterial Infections of the Eyes
A number of bacteria are able to cause infection when introduced to the mucosa of the eye. In general, bacterial eye infections can lead to inflammation, irritation, and discharge, but they vary in severity. Some are typically short-lived, and others can become chronic and lead to permanent eye damage. Prevention requires limiting exposure to contagious pathogens. When infections do occur, prompt treatment with antibiotics can often limit or prevent permanent damage. [\[link\]](#) summarizes the characteristics of some common bacterial infections of the eyes.

Bacterial Infections of the Eyes				
Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Acute bacterial conjunctivitis	<i>Haemophilus influenzae</i>	Inflammation of conjunctiva with purulent discharge	Exposure to secretions from infected individuals	Broad-spectrum topical antibiotics
Bacterial keratitis	<i>Staphylococcus epidermidis</i> , <i>Pseudomonas aeruginosa</i>	Redness and irritation of eye, blurred vision, sensitivity to light; progressive corneal scarring, which can lead to blindness	Exposure to pathogens on contaminated contact lenses	Antibiotic eye drops (e.g., with fluoroquinolones)
Neonatal conjunctivitis	<i>Chlamydia trachomatis</i> , <i>Neisseria gonorrhoeae</i>	Inflammation of conjunctiva, purulent discharge, scarring and perforation of cornea; may lead to blindness	Neonate exposed to pathogens in birth canal of mother with chlamydia or gonorrhea	Erythromycin
Trachoma (granular conjunctivitis)	<i>C. trachomatis</i>	Chronic conjunctivitis, trichiasis, scarring, blindness	Contact with infected individuals or contaminated fomites; transmission by eye-seeking flies	Azithromycin

Key Concepts and Summary

- *Staphylococcus* and *Streptococcus* cause many different types of skin infections, many of which occur when bacteria breach the skin barrier through a cut or wound.
- *S. aureus* are frequently associated with purulent skin infections that manifest as **folliculitis**, **furuncles**, or **carbuncles**. *S. aureus* is also a leading cause of staphylococcal scalded skin syndrome (SSSS).
- *S. aureus* is generally drug resistant and current MRSA strains are resistant to a wide range of antibiotics.
- Community-acquired and hospital-acquired staphylococcal infections are an ongoing problem because many people are asymptomatic carriers.
- **Group A streptococci (GAS)**, *S. pyogenes*, is often responsible for cases of **cellulitis**, **erysipelas**, and **erythema nodosum**. GAS are also one of many possible causes of **necrotizing fasciitis**.
- *P. aeruginosa* is often responsible for infections of the skin and eyes, including wound and burn infections, **hot tub rash**, **otitis externa**, and bacterial **keratitis**.
- **Acne** is a common skin condition that can become more inflammatory when *Propionibacterium acnes* infects hair follicles and pores clogged with dead skin cells and sebum.
- Cutaneous **anthrax** occurs when *Bacillus anthracis* breaches the skin barrier. The infection results in a localized black **eschar** on skin. Anthrax can be fatal if *B. anthracis* spreads to the bloodstream.
- Common bacterial **conjunctivitis** is often caused by *Haemophilus influenzae* and usually resolves on its own in a few days. More serious forms of conjunctivitis include gonococcal **ophthalmia neonatorum**, **inclusion conjunctivitis** (chlamydial), and **trachoma**, all of which can lead to blindness if untreated.
- **Keratitis** is frequently caused by *Staphylococcus epidermidis* and/or *Pseudomonas aeruginosa*, especially among contact lens users, and can lead to blindness.
- Biofilms complicate the treatment of wound and eye infections because pathogens living in biofilms can be difficult to treat and eliminate.

Multiple Choice

Exercise:

Problem: *Staphylococcus aureus* is most often associated with being

- a. coagulase-positive.
- b. coagulase-negative.
- c. catalase-negative.
- d. gram-negative

Solution:

A

Exercise:

Problem: M protein is produced by

- a. *Pseudomonas aeruginosa*
- b. *Staphylococcus aureus*
- c. *Propionibacterium acnes*
- d. *Streptococcus pyogenes*

Solution:

D

Exercise:

Problem:

_____ is a major cause of preventable blindness that can be reduced through improved sanitation.

- a. Ophthalmia neonatorum
- b. Keratitis
- c. Trachoma
- d. Cutaneous anthrax

Solution:

C

Exercise:

Problem:

Which species is frequently associated with nosocomial infections transmitted via medical devices inserted into the body?

- a. *Staphylococcus epidermidis*
- b. *Streptococcus pyogenes*
- c. *Propionibacterium acnes*
- d. *Bacillus anthracis*

Solution:

A

Fill in the Blank

Exercise:

Problem: A purulent wound produces _____.

Solution:

pus

Short Answer

Exercise:

Problem: How are leukocidins associated with pus production?

Exercise:

Problem:

What is a good first test to distinguish streptococcal infections from staphylococcal infections?

Viral Infections of the Skin and Eyes

LEARNING OBJECTIVES

- Identify the most common viruses associated with infections of the skin and eyes
- Compare the major characteristics of specific viral diseases affecting the skin and eyes

Until recently, it was thought that the normal microbiota of the body consisted primarily of bacteria and some fungi. However, in addition to bacteria, the skin is colonized by viruses, and recent studies suggest that Papillomaviridae, Polyomaviridae and Circoviridae also contribute to the normal skin microbiota. However, some viruses associated with skin are pathogenic, and these viruses can cause diseases with a wide variety of presentations.

Numerous types of viral infections cause rashes or lesions on the skin; however, in many cases these skin conditions result from infections that originate in other body systems. In this chapter, we will limit the discussion to viral skin infections that use the skin as a portal of entry. Later chapters will discuss viral infections such as chickenpox, measles, and rubella—diseases that cause skin rashes but invade the body through portals of entry other than the skin.

Papillomas

Papillomas (warts) are the expression of common skin infections by human papillomavirus (HPV) and are transmitted by direct contact. There are many

types of HPV, and they lead to a variety of different presentations, such as common warts, plantar warts, flat warts, and filiform warts. HPV can also cause sexually-transmitted genital warts, which will be discussed in [Urogenital System Infections](#). Vaccination is available for some strains of HPV.

Common warts tend to develop on fingers, the backs of hands, and around nails in areas with broken skin. In contrast, plantar warts (also called foot warts) develop on the sole of the foot and can grow inwards, causing pain and pressure during walking. Flat warts can develop anywhere on the body, are often numerous, and are relatively smooth and small compared with other wart types. Filiform warts are long, threadlike warts that grow quickly.

In some cases, the immune system may be strong enough to prevent warts from forming or to eradicate established warts. However, treatment of established warts is typically required. There are many available treatments for warts, and their effectiveness varies. Common warts can be frozen off with liquid nitrogen. Topical applications of salicylic acid may also be effective. Other options are electrosurgery (burning), curettage (cutting), excision, painting with cantharidin (which causes the wart to die so it can more easily be removed), laser treatments, treatment with bleomycin, chemical peels, and immunotherapy ([\[link\]](#)).



(a)



(b)

Warts can vary in shape and in location. (a) Multiple plantar warts have grown on this toe. (b) A filiform wart has grown on this eyelid.

Oral Herpes

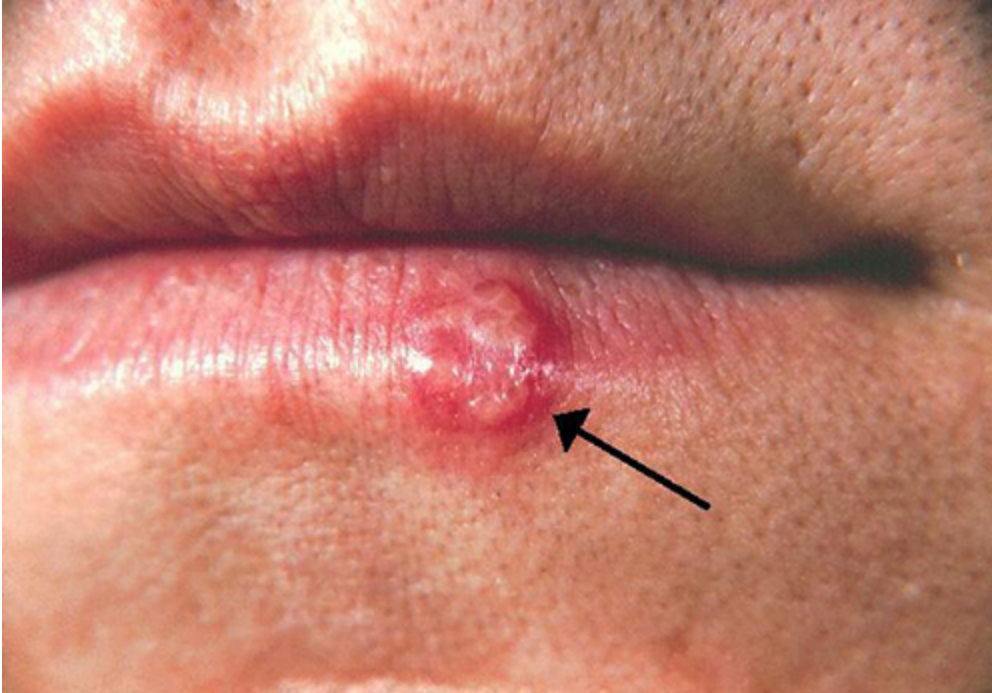
Another common skin virus is herpes simplex virus (HSV). HSV has historically been divided into two types, HSV-1 and HSV-2. HSV-1 is typically transmitted by direct oral contact between individuals, and is usually associated with **oral herpes**. HSV-2 is usually transmitted sexually and is typically associated with genital herpes. However, both HSV-1 and HSV-2 are capable of infecting any mucous membrane, and the incidence of genital HSV-1 and oral HSV-2 infections has been increasing in recent years. In this chapter, we will limit our discussion to infections caused by HSV-1; HSV-2 and genital herpes will be discussed in [Urogenital System Infections](#).

Infection by HSV-1 commonly manifests as cold sores or fever blisters, usually on or around the lips ([link](#)). HSV-1 is highly contagious, with some studies suggesting that up to 65% of the US population is infected; however, many infected individuals are asymptomatic.[\[footnote\]](#) Moreover,

the virus can be latent for long periods, residing in the trigeminal nerve ganglia between recurring bouts of symptoms. Recurrence can be triggered by stress or environmental conditions (systemic or affecting the skin). When lesions are present, they may blister, break open, and crust. The virus can be spread through direct contact, even when a patient is asymptomatic. Wald, A., and Corey, L. "Persistence in the Population: Epidemiology, Transmission." In: A. Arvin, G. Campadelli-Fiume, E. Mocarski et al. *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis*. Cambridge: Cambridge University Press, 2007. <http://www.ncbi.nlm.nih.gov/books/NBK47447/>. Accessed Sept 14, 2016.

While the lips, mouth, and face are the most common sites for HSV-1 infections, lesions can spread to other areas of the body. Wrestlers and other athletes involved in contact sports may develop lesions on the neck, shoulders, and trunk. This condition is often called herpes gladiatorum. Herpes lesions that develop on the fingers are often called herpetic whitlow.

HSV-1 infections are commonly diagnosed from their appearance, although laboratory testing can confirm the diagnosis. There is no cure, but antiviral medications such as acyclovir, penciclovir, famciclovir, and valacyclovir are used to reduce symptoms and risk of transmission. Topical medications, such as creams with *n*-docosanol and penciclovir, can also be used to reduce symptoms such as itching, burning, and tingling.



This cold sore was caused by HSV-1. (credit: Centers for Disease Control and Prevention)

Note:

- What are the most common sites for the appearance of herpetic lesions?

Roseola and Fifth Disease

The viral diseases **roseola** and **fifth disease** are somewhat similar in terms of their presentation, but they are caused by different viruses. Roseola, sometimes called roseola infantum or exanthem subitum (“sudden rash”), is a mild viral infection usually caused by human herpesvirus-6 (HHV-6) and

occasionally by HHV-7. It is spread via direct contact with the saliva or respiratory secretions of an infected individual, often through droplet aerosols. Roseola is very common in children, with symptoms including a runny nose, a sore throat, and a cough, along with (or followed by) a high fever (39.4 °C). About three to five days after the fever subsides, a rash may begin to appear on the chest and abdomen. The rash, which does not cause discomfort, initially forms characteristic macules that are flat or papules that are firm and slightly raised; some macules or papules may be surrounded by a white ring. The rash may eventually spread to the neck and arms, and sometimes continues to spread to the face and legs. The diagnosis is generally made based upon observation of the symptoms. However, it is possible to perform serological tests to confirm the diagnosis. While treatment may be recommended to control the fever, the disease usually resolves without treatment within a week after the fever develops. For individuals at particular risk, such as those who are immunocompromised, the antiviral medication ganciclovir may be used.

Fifth disease (also known as erythema infectiosum) is another common, highly contagious illness that causes a distinct rash that is critical to diagnosis. Fifth disease is caused by parvovirus B19, and is transmitted by contact with respiratory secretions from an infected individual. Infection is more common in children than adults. While approximately 20% of individuals will be asymptomatic during infection, [\[footnote\]](#) others will exhibit cold-like symptoms (headache, fever, and upset stomach) during the early stages when the illness is most infectious. Several days later, a distinct red facial rash appears, often called “slapped cheek” rash ([\[link\]](#)). Within a few days, a second rash may appear on the arms, legs, chest, back, or buttocks. The rash may come and go for several weeks, but usually disappears within seven to twenty-one days, gradually becoming lacy in appearance as it recedes.

Centers for Disease Control and Prevention. “Fifth Disease.”

<http://www.cdc.gov/parvovirusb19/fifth-disease.html>. Accessed Sept 14, 2016.

In children, the disease usually resolves on its own without medical treatment beyond symptom relief as needed. Adults may experience different and possibly more serious symptoms. Many adults with fifth

disease do not develop any rash, but may experience joint pain and swelling that lasts several weeks or months. Immunocompromised individuals can develop severe anemia and may need blood transfusions or immune globulin injections. While the rash is the most important component of diagnosis (especially in children), the symptoms of fifth disease are not always consistent. Serological testing can be conducted for confirmation.



(a)



(b)

(a) Roseola, a mild viral infection common in young children, generally begins with symptoms similar to a cold, followed by a pink, patchy rash that starts on the trunk and spreads outward. (b) Fifth disease exhibits similar symptoms in children, except for the distinctive “slapped cheek” rash that originates on the face.

Note:

- Identify at least one similarity and one difference between roseola and fifth disease.

Viral Conjunctivitis

Like bacterial conjunctivitis viral infections of the eye can cause inflammation of the conjunctiva and discharge from the eye. However, **viral conjunctivitis** tends to produce a discharge that is more watery than the thick discharge associated with bacterial conjunctivitis. The infection is contagious and can easily spread from one eye to the other or to other individuals through contact with eye discharge.

Viral conjunctivitis is commonly associated with colds caused by adenoviruses; however, other viruses can also cause conjunctivitis. If the causative agent is uncertain, eye discharge can be tested to aid in diagnosis. Antibiotic treatment of viral conjunctivitis is ineffective, and symptoms usually resolve without treatment within a week or two.

Herpes Keratitis

Herpes infections caused by HSV-1 can sometimes spread to the eye from other areas of the body, which may result in keratoconjunctivitis. This condition, generally called **herpes keratitis** or herpetic keratitis, affects the conjunctiva and cornea, causing irritation, excess tears, and sensitivity to light. Deep lesions in the cornea may eventually form, leading to blindness. Because keratitis can have numerous causes, laboratory testing is necessary to confirm the diagnosis when HSV-1 is suspected; once confirmed, antiviral medications may be prescribed.

Note:

Viral Infections of the Skin and Eyes

A number of viruses can cause infections via direct contact with skin and eyes, causing signs and symptoms ranging from rashes and lesions to warts and conjunctivitis. All of these viral diseases are contagious, and while some are more common in children (fifth disease and roseola), others are prevalent in people of all ages (oral herpes, viral conjunctivitis, papillomas). In general, the best means of prevention is avoiding contact with infected individuals. Treatment may require antiviral medications; however, several of these conditions are mild and typically resolve without treatment. [\[link\]](#) summarizes the characteristics of some common viral infections of the skin and eyes.

Viral Infections of the Skin and Eyes				
Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Fifth disease	Parvovirus B19	May have initial cold-like symptoms; "slapped cheek" rash	Highly contagious via respiratory secretions of infected individuals	None
Herpes keratitis	Herpes simplex virus 1 (HSV-1)	Inflammation of conjunctiva and cornea; irritation, excess tears, sensitivity to light; lesions in cornea leading to blindness	Direct eye contact with discharge from herpes lesions elsewhere in the body or from another infected individual	Acyclovir, ganciclovir, famciclovir, valacyclovir
Oral herpes	Herpes simplex virus 1 (HSV-1)	May cause initial systemic symptoms; cold sores	Highly contagious via direct contact with infected individuals	Acyclovir, penciclovir, famciclovir, valacyclovir
Papillomas	Human papillomavirus (HPV)	Common warts, plantar warts, flat warts, filiform warts, and others	Contact with infected individuals	Topical salicylic acid, cantharidin
Roseola (roseola infantum, exanthem subitum)	Human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7)	Initial cold-like symptoms with high fever, followed by a macular or papular rash three to five days later	Spread by viral and respiratory secretions of infected individuals	Typically none; ganciclovir for immunocompromised patients
Viral conjunctivitis	Adenoviruses and others	Inflammation of the conjunctiva; watery, nonpurulent discharge	Associated with common cold; contagious via contact with eye discharge	None

Key Concepts and Summary

- **Papillomas** (warts) are caused by human papillomaviruses.
- **Herpes simplex virus** (especially HSV-1) mainly causes **oral herpes**, but lesions can appear on other areas of the skin and mucous membranes.
- **Roseola** and **fifth disease** are common viral illnesses that cause skin rashes; roseola is caused by HHV-6 and HHV-7 while fifth disease is caused by parvovirus 19.
- **Viral conjunctivitis** is often caused by adenoviruses and may be associated with the common cold. **Herpes keratitis** is caused by herpesviruses that spread to the eye.

Multiple Choice

Exercise:

Problem: Warts are caused by

- a. human papillomavirus.
- b. herpes simplex virus.
- c. adenoviruses.
- d. parvovirus B19.

Solution:

A

Exercise:

Problem:

Which of these viruses can spread to the eye to cause a form of keratitis?

- a. human papillomavirus
- b. herpes simplex virus 1
- c. parvovirus 19
- d. circoviruses

Solution:

B

Exercise:

Problem: Cold sores are associated with:

- a. human papillomavirus
- b. roseola
- c. herpes simplex viruses
- d. human herpesvirus 6

Solution:

C

Exercise:

Problem:

Which disease is usually self-limiting but is most commonly treated with ganciclovir if medical treatment is needed?

- a. roseola
- b. oral herpes
- c. papillomas
- d. viral conjunctivitis

Solution:

A

Exercise:

Problem: Adenoviruses can cause:

- a. viral conjunctivitis
- b. herpetic conjunctivitis
- c. papillomas
- d. oral herpes

Solution:

A

Fill in the Blank

Exercise:

Problem: Human herpesvirus 6 is the causative agent of _____.

Solution:

roseola

Short Answer

Exercise:

Problem: Compare and contrast bacterial and viral conjunctivitis.

Mycoses of the Skin

LEARNING OBJECTIVES

- Identify the most common fungal pathogens associated with cutaneous and subcutaneous mycoses
- Compare the major characteristics of specific fungal diseases affecting the skin

Many fungal infections of the skin involve fungi that are found in the normal skin microbiota. Some of these fungi can cause infection when they gain entry through a wound; others mainly cause opportunistic infections in immunocompromised patients. Other fungal pathogens primarily cause infection in unusually moist environments that promote fungal growth; for example, sweaty shoes, communal showers, and locker rooms provide excellent breeding grounds that promote the growth and transmission of fungal pathogens.

Fungal infections, also called mycoses, can be divided into classes based on their invasiveness. Mycoses that cause superficial infections of the epidermis, hair, and nails, are called **cutaneous mycoses**. Mycoses that penetrate the epidermis and the dermis to infect deeper tissues are called **subcutaneous mycoses**. Mycoses that spread throughout the body are called **systemic mycoses**.

Tineas

A group of cutaneous mycoses called **tineas** are caused by **dermatophytes**, fungal molds that require keratin, a protein found in skin, hair, and nails, for

growth. There are three genera of dermatophytes, all of which can cause cutaneous mycoses: *Trichophyton*, *Epidermophyton*, and *Microsporum*. Tineas on most areas of the body are generally called **ringworm**, but tineas in specific locations may have distinctive names and symptoms (see [\[link\]](#) and [\[link\]](#)). Keep in mind that these names—even though they are Latinized—refer to locations on the body, not causative organisms. Tineas can be caused by different dermatophytes in most areas of the body.

Some Common Tineas and Location on the Body	
Tinea corporis (ringworm)	Body
Tinea capitis (ringworm)	Scalp
Tinea pedis (athlete's foot)	Feet
Tinea barbae (barber's itch)	Beard
Tinea cruris (jock itch)	Groin
Tinea unguium (onychomycosis)	Toenails, fingernails



(a)



(b)



(c)

Tineas are superficial cutaneous mycoses and are common. (a) *Tinea barbae* (barber's itch) occurs on the lower face. (b) *Tinea pedis* (athlete's foot) occurs on the feet, causing itching, burning, and dry, cracked skin between the toes. (c) A close-up view of *tinea corporis* (ringworm) caused by *Trichophyton mentagrophytes*. (credit a, c: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Al Hasan M, Fitzgerald SM, Saoudian M, Krishnaswamy G)

Dermatophytes are commonly found in the environment and in soils and are frequently transferred to the skin via contact with other humans and animals. Fungal spores can also spread on hair. Many dermatophytes grow well in moist, dark environments. For example, **tinea pedis** (athlete's foot) commonly spreads in public showers, and the causative fungi grow well in the dark, moist confines of sweaty shoes and socks. Likewise, **tinea cruris** (jock itch) often spreads in communal living environments and thrives in warm, moist undergarments.

Tineas on the body (**tinea corporis**) often produce lesions that grow radially and heal towards the center. This causes the formation of a red ring, leading to the misleading name of ringworm recall the Clinical Focus case in [The Eukaryotes of Microbiology](#).

Several approaches may be used to diagnose tineas. A Wood's lamp (also called a black lamp) with a wavelength of 365 nm is often used. When directed on a tinea, the ultraviolet light emitted from the Wood's lamp causes the fungal elements (spores and hyphae) to fluoresce. Direct microscopic evaluation of specimens from skin scrapings, hair, or nails can also be used to detect fungi. Generally, these specimens are prepared in a wet mount using a potassium hydroxide solution (10%–20% aqueous KOH), which dissolves the keratin in hair, nails, and skin cells to allow for visualization of the hyphae and fungal spores. The specimens may be grown on Sabouraud dextrose CC (chloramphenicol/cyclohexamide), a selective agar that supports dermatophyte growth while inhibiting the growth of bacteria and saprophytic fungi ([\[link\]](#)). Macroscopic colony

morphology is often used to initially identify the genus of the dermatophyte; identification can be further confirmed by visualizing the microscopic morphology using either a slide culture or a sticky tape prep stained with lactophenol cotton blue.

Various antifungal treatments can be effective against tinea. Allylamine ointments that include terbinafine are commonly used; miconazole and clotrimazole are also available for topical treatment, and griseofulvin is used orally.



To diagnose tinea, the dermatophytes may be grown on a Sabouraud dextrose CC agar plate. This culture contains a strain of *Trichophyton rubrum*, one of the most common causes of tinea on various parts of the body. (credit: Centers for Disease Control and Prevention)

Note:

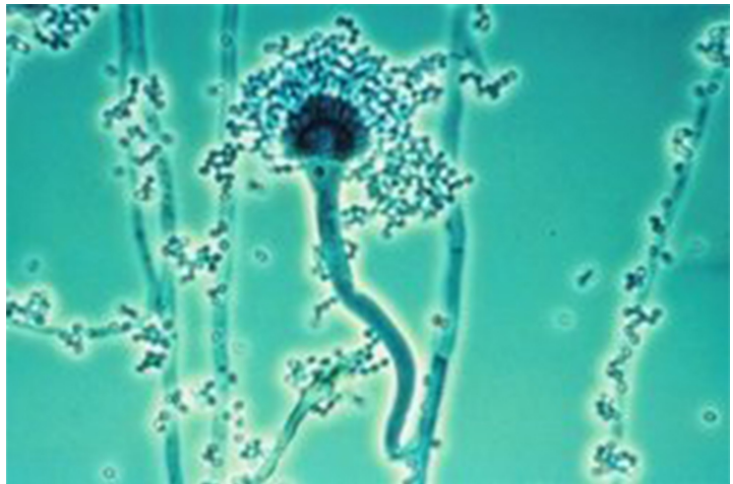
- Why are tinea, caused by fungal molds, often called ringworm?

Cutaneous Aspergillosis

Another cause of cutaneous mycoses is *Aspergillus*, a genus consisting of molds of many different species, some of which cause a condition called aspergillosis. Primary cutaneous aspergillosis, in which the infection begins in the skin, is rare but does occur. More common is secondary cutaneous aspergillosis, in which the infection begins in the respiratory system and disseminates systemically. Both primary and secondary cutaneous aspergillosis result in distinctive eschars that form at the site or sites of infection ([\[link\]](#)). Pulmonary aspergillosis will be discussed more thoroughly in [Respiratory Mycoses](#)).



(a)



(b)

(a) Eschar on a patient with secondary cutaneous aspergillosis. (b)

Micrograph showing a conidiophore of *Aspergillus*. (credit a: modification of work by Santiago M, Martinez JH, Palermo C, Figueroa C, Torres O, Trinidad R, Gonzalez E, Miranda Mde L, Garcia M, Villamarzo G; credit b: modification of work by U.S. Department of Health and Human Services)

Primary cutaneous aspergillosis usually occurs at the site of an injury and is most often caused by *Aspergillus fumigatus* or *Aspergillus flavus*. It is usually reported in patients who have had an injury while working in an agricultural or outdoor environment. However, opportunistic infections can also occur in health-care settings, often at the site of intravenous catheters, venipuncture wounds, or in association with burns, surgical wounds, or occlusive dressing. After candidiasis, aspergillosis is the second most common hospital-acquired fungal infection and often occurs in immunocompromised patients, who are more vulnerable to opportunistic infections.

Cutaneous aspergillosis is diagnosed using patient history, culturing, histopathology using a skin biopsy. Treatment involves the use of antifungal medications such as voriconazole (preferred for invasive aspergillosis), itraconazole, and amphotericin B if itraconazole is not effective. For immunosuppressed individuals or burn patients, medication may be used and surgical or immunotherapy treatments may be needed.

Note:

- Identify the sources of infection for primary and secondary cutaneous aspergillosis.

Candidiasis of the Skin and Nails

Candida albicans and other yeasts in the genus *Candida* can cause skin infections referred to as cutaneous candidiasis. *Candida* spp. are sometimes responsible for **intertrigo**, a general term for a rash that occurs in a skin fold, or other localized rashes on the skin. *Candida* can also infect the nails, causing them to become yellow and harden ([link](#)).



(a)



(b)



(c)

(a) This red, itchy rash is the result of cutaneous candidiasis, an opportunistic infection of the skin caused by the yeast *Candida albicans*. (b) Fungal infections of the nail (tinea unguium) can be caused by dermatophytes or *Candida* spp. The nail becomes yellow, brittle, and prone to breaking. This condition is relatively common among adults. (c) *C. albicans* growing on Sabouraud dextrose agar. (credit a: modification of work by U.S. Department of Veterans Affairs; credit c: modification of work by Centers for Disease Control and Prevention)

Candidiasis of the skin and nails is diagnosed through clinical observation and through culture, Gram stain, and KOH wet mounts. Susceptibility testing for anti-fungal agents can also be done. Cutaneous candidiasis can be treated with topical or systemic azole antifungal medications. Because candidiasis can become invasive, patients suffering from HIV/AIDS, cancer, or other conditions that compromise the immune system may benefit from preventive treatment. Azoles, such as clotrimazole, econazole, fluconazole, ketoconazole, and miconazole; nystatin; terbinafine; and naftifine may be used for treatment. Long-term treatment with medications

such as itraconazole or ketoconazole may be used for chronic infections. Repeat infections often occur, but this risk can be reduced by carefully following treatment recommendations, avoiding excessive moisture, maintaining good health, practicing good hygiene, and having appropriate clothing (including footwear).

Candida also causes infections in other parts of the body besides the skin. These include vaginal yeast infections (see [Fungal Infections of the Reproductive System](#)) and oral thrush (see [Microbial Diseases of the Mouth and Oral Cavity](#)).

Note:

- What are the signs and symptoms of candidiasis of the skin and nails?

Sporotrichosis

Whereas cutaneous mycoses are superficial, subcutaneous mycoses can spread from the skin to deeper tissues. In temperate regions, the most common subcutaneous mycosis is a condition called **sporotrichosis**, caused by the fungus *Sporothrix schenckii* and commonly known as rose gardener's disease or rose thorn disease (recall [Case in Point: Every Rose Has Its Thorn](#)). Sporotrichosis is often contracted after working with soil, plants, or timber, as the fungus can gain entry through a small wound such as a thorn-prick or splinter. Sporotrichosis can generally be avoided by wearing gloves and protective clothing while gardening and promptly cleaning and disinfecting any wounds sustained during outdoor activities.

Sporothrix infections initially present as small ulcers in the skin, but the fungus can spread to the lymphatic system and sometimes beyond. When the infection spreads, nodules appear, become necrotic, and may ulcerate. As more lymph nodes become affected, abscesses and ulceration may develop over a larger area (often on one arm or hand). In severe cases, the

infection may spread more widely throughout the body, although this is relatively uncommon.

Sporothrix infection can be diagnosed based upon histologic examination of the affected tissue. Its macroscopic morphology can be observed by culturing the mold on potato dextrose agar, and its microscopic morphology can be observed by staining a slide culture with lactophenol cotton blue. Treatment with itraconazole is generally recommended.

Note:

- Describe the progression of a *Sporothrix schenckii* infection.

Note:

Mycoses of the Skin

Cutaneous mycoses are typically opportunistic, only able to cause infection when the skin barrier is breached through a wound. Tineas are the exception, as the dermatophytes responsible for tineas are able to grow on skin, hair, and nails, especially in moist conditions. Most mycoses of the skin can be avoided through good hygiene and proper wound care. Treatment requires antifungal medications. [\[link\]](#) summarizes the characteristics of some common fungal infections of the skin.

Mycoses of the Skin				
Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Aspergillosis (cutaneous)	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i>	Distinctive eschars at site(s) of infection	Entry via wound (primary cutaneous aspergillosis) or via the respiratory system (secondary cutaneous aspergillosis); commonly a hospital-acquired infection	Itraconazole, voriconazole, amphotericin B
Candidiasis (cutaneous)	<i>Candida albicans</i>	Intertrigo, localized rash, yellowing of nails	Overgrowth of normal skin microbiota, especially in moist, dark areas	Azoles
Sporotrichosis (rose gardener's disease)	<i>Sporothrix schenckii</i>	Subcutaneous ulcers and abscesses; may spread to a large area, e.g., hand or arm	Entry via thorn prick or other wound	Itraconazole
Tineas	<i>Trichophyton</i> spp., <i>Epidermophyton</i> spp., <i>Microsporum</i> spp.	Itchy, ring-like lesions (ringworm) at sites of infection	Contact with dermatophytic fungi, especially in warm, moist environments conducive to fungal growth	Terbinafine, miconazole, clotrimazole, griseofulvin

Key Concepts and Summary

- **Mycoses** can be **cutaneous**, **subcutaneous**, or **systemic**.
- Common cutaneous mycoses include **tineas** caused by **dermatophytes** of the genera *Trichophyton*, *Epidermophyton*, and *Microsporum*. **Tinea corporis** is called **ringworm**. Tineas on other parts of the body have names associated with the affected body part.
- **Aspergillosis** is a fungal disease caused by molds of the genus *Aspergillus*. Primary cutaneous aspergillosis enters through a break in the skin, such as the site of an injury or a surgical wound; it is a common hospital-acquired infection. In secondary cutaneous aspergillosis, the fungus enters via the respiratory system and disseminates systemically, manifesting in lesions on the skin.
- The most common subcutaneous mycosis is **sporotrichosis** (rose gardener's disease), caused by *Sporothrix schenckii*.

- Yeasts of the genus *Candida* can cause opportunistic infections of the skin called **candidiasis**, producing **intertrigo**, localized rashes, or yellowing of the nails.

Multiple Choice

Exercise:

Problem:

_____ is a superficial fungal infection found on the head.

- a. Tinea cruris
- b. Tinea capitis
- c. Tinea pedis
- d. Tinea corporis

Solution:

B

Exercise:

Problem:

For what purpose would a health-care professional use a Wood's lamp for a suspected case of ringworm?

- a. to prevent the rash from spreading
- b. to kill the fungus
- c. to visualize the fungus
- d. to examine the fungus microscopically

Solution:

C

Exercise:

Problem: Sabouraud dextrose agar CC is selective for:

- a. all fungi
- b. non-saprophytic fungi
- c. bacteria
- d. viruses

Solution:

B

Exercise:

Problem: The first-line recommended treatment for sporotrichosis is:

- a. itraconazole
- b. clindamycin
- c. amphotericin
- d. nystatin

Solution:

A

Fill in the Blank

Exercise:

Problem:

The most common subcutaneous mycosis in temperate regions is _____.

Solution:

sporotrichosis

Short Answer

Exercise:

Problem: What yeasts commonly cause opportunistic infections?

Critical Thinking

Exercise:

Problem:

What steps might you recommend to a patient for reducing the risk of developing a fungal infection of the toenails?

Protozoan and Helminthic Infections of the Skin and Eyes

LEARNING OBJECTIVES

- Identify two parasites that commonly cause infections of the skin and eyes
- Identify the major characteristics of specific parasitic diseases affecting the skin and eyes

Many parasitic protozoans and helminths use the skin or eyes as a portal of entry. Some may physically burrow into the skin or the mucosa of the eye; others breach the skin barrier by means of an insect bite. Still others take advantage of a wound to bypass the skin barrier and enter the body, much like other opportunistic pathogens. Although many parasites enter the body through the skin, in this chapter we will limit our discussion to those for which the skin or eyes are the primary site of infection. Parasites that enter through the skin but travel to a different site of infection will be covered in other chapters. In addition, we will limit our discussion to microscopic parasitic infections of the skin and eyes. Macroscopic parasites such as lice, scabies, mites, and ticks are beyond the scope of this text.

***Acanthamoeba* Infections**

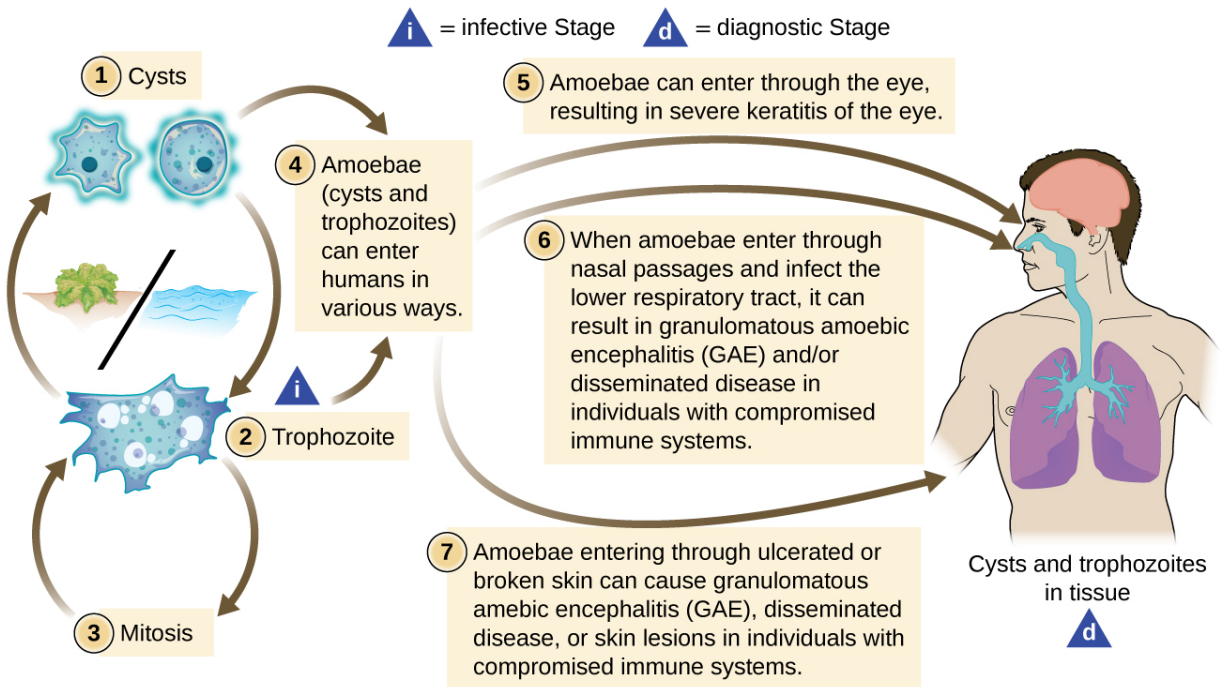
Acanthamoeba is a genus of free-living protozoan amoebae that are common in soils and unchlorinated bodies of fresh water. (This is one reason why some swimming pools are treated with chlorine.) The genus contains a few parasitic species, some of which can cause infections of the eyes, skin, and nervous system. Such infections can sometimes travel and affect other body systems. Skin infections may manifest as abscesses,

ulcers, and nodules. When *acanthamoebae* infect the eye, causing inflammation of the cornea, the condition is called ***Acanthamoeba* keratitis**. [\[link\]](#) illustrates the *Acanthamoeba* life cycle and various modes of infection.

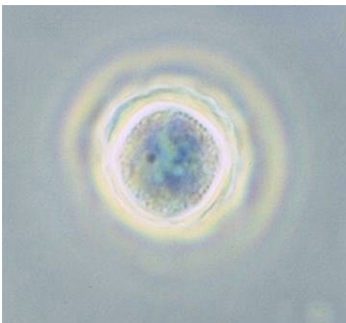
While *Acanthamoeba* keratitis is initially mild, it can lead to severe corneal damage, vision impairment, or even blindness if left untreated. Similar to eye infections involving *P. aeruginosa*, *Acanthamoeba* poses a much greater risk to wearers of contact lenses because the amoeba can thrive in the space between contact lenses and the cornea. Prevention through proper contact lens care is important. Lenses should always be properly disinfected prior to use, and should never be worn while swimming or using a hot tub.

Acanthamoeba can also enter the body through other pathways, including skin wounds and the respiratory tract. It usually does not cause disease except in immunocompromised individuals; however, in rare cases, the infection can spread to the nervous system, resulting in a usually fatal condition called granulomatous amoebic encephalitis (GAE) (see [Fungal and Parasitic Diseases of the Nervous System](#)). Disseminated infections, lesions, and *Acanthamoeba* keratitis can be diagnosed by observing symptoms and examining patient samples under the microscope to view the parasite. Skin biopsies may be used.

Acanthamoeba keratitis is difficult to treat, and prompt treatment is necessary to prevent the condition from progressing. The condition generally requires three to four weeks of intensive treatment to resolve. Common treatments include topical antiseptics (e.g., polyhexamethylene biguanide, chlorhexidine, or both), sometimes with painkillers or corticosteroids (although the latter are controversial because they suppress the immune system, which can worsen the infection). Azoles are sometimes prescribed as well. Advanced cases of keratitis may require a corneal transplant to prevent blindness.



Acanthamoeba spp. are waterborne parasites very common in unchlorinated aqueous environments. As shown in this life cycle, *Acanthamoeba* cysts and trophozoites are both capable of entering the body through various routes, causing infections of the eye, skin, and central nervous system. (credit: modification of work by Centers for Disease Control and Prevention)



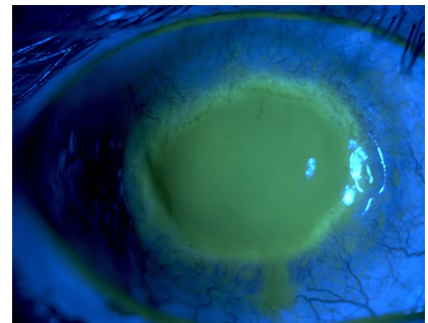
Acanthamoeba cyst

(a)



Acanthamoeba trophozoite

(b)



(c)

(a) An *Acanthamoeba* cyst. (b) An *Acanthamoeba* trophozoite (c) The eye of a patient with *Acanthamoeba* keratitis. The fluorescent color, which is due to sodium fluorescein application, highlights significant damage to the cornea and vascularization of the surrounding conjunctiva. (credit a: modification of work by Centers for Disease Control and Prevention; credit b, c: modification of work by Jacob Lorenzo-Morales, Naveed A Kahn and Julia Walochnik)

Note:

- How are *Acanthamoeba* infections acquired?

Loiasis

The helminth *Loa loa*, also known as the African eye worm, is a nematode that can cause **loiasis**, a disease endemic to West and Central Africa ([\[link\]](#)). The disease does not occur outside that region except when carried by travelers. There is evidence that individual genetic differences affect susceptibility to developing loiasis after infection by the *Loa loa* worm. Even in areas in which *Loa loa* worms are common, the disease is generally found in less than 30% of the population.[\[footnote\]](#) It has been suggested that travelers who spend time in the region may be somewhat more susceptible to developing symptoms than the native population, and the presentation of infection may differ.[\[footnote\]](#)

Garcia, A.. et al. “Genetic Epidemiology of Host Predisposition Microfilaraemia in Human Loiasis.” *Tropical Medicine and International Health* 4 (1999) 8:565–74. <http://www.ncbi.nlm.nih.gov/pubmed/10499080>. Accessed Sept 14, 2016.

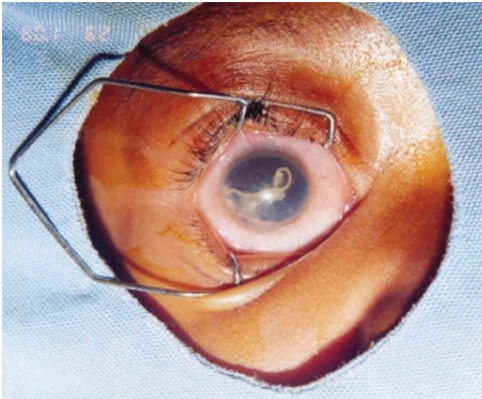
Spinello, A., et al. “Imported *Loa loa* Filariasis: Three Cases and a Review of Cases Reported in Non-Endemic Countries in the Past 25 Years.”

International Journal of Infectious Disease 16 (2012) 9: e649–e662. DOI: <http://dx.doi.org/10.1016/j.ijid.2012.05.1023>.

The parasite is spread by deerflies (genus *Chrysops*), which can ingest the larvae from an infected human via a blood meal ([\[link\]](#)). When the deerfly bites other humans, it deposits the larvae into their bloodstreams. After about five months in the human body, some larvae develop into adult worms, which can grow to several centimeters in length and live for years in the subcutaneous tissue of the host.

The name “eye worm” alludes to the visible migration of worms across the conjunctiva of the eye. Adult worms live in the subcutaneous tissues and can travel at about 1 cm per hour. They can often be observed when migrating through the eye, and sometimes under the skin; in fact, this is generally how the disease is diagnosed. It is also possible to test for antibodies, but the presence of antibodies does not necessarily indicate a current infection; it only means that the individual was exposed at some time. Some patients are asymptomatic, but in others the migrating worms can cause fever and areas of allergic inflammation known as Calabar swellings. Worms migrating through the conjunctiva can cause temporary eye pain and itching, but generally there is no lasting damage to the eye. Some patients experience a range of other symptoms, such as widespread itching, hives, and joint and muscle pain.

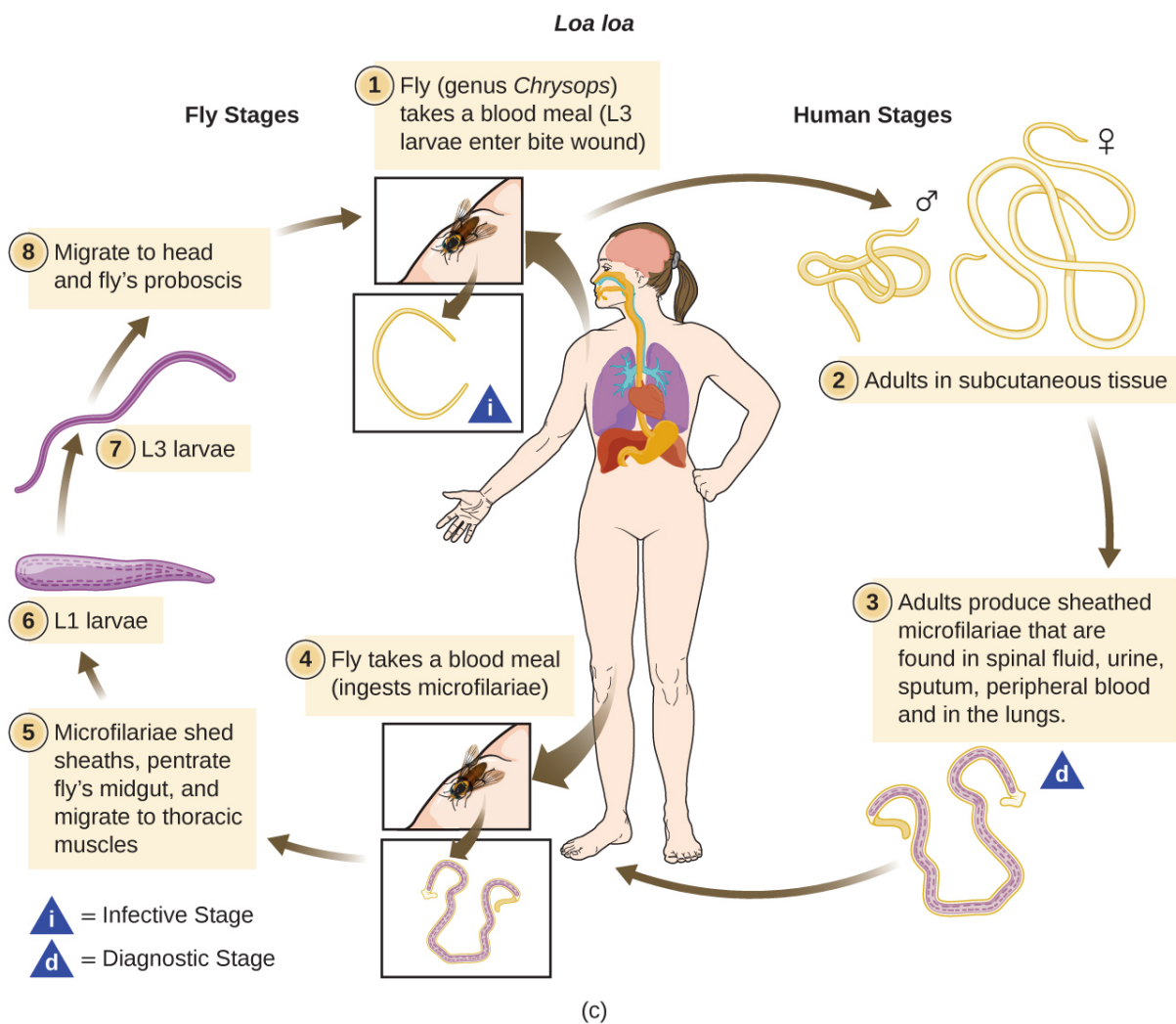
Worms can be surgically removed from the eye or the skin, but this treatment only relieves discomfort; it does not cure the infection, which involves many worms. The preferred treatment is diethylcarbamazine, but this medication produces severe side effects in some individuals, such as brain inflammation and possible death in patients with heavy infections. Albendazole is also sometimes used if diethylcarbamazine is not appropriate or not successful. If left untreated for many years, loiasis can damage the kidneys, heart, and lungs, though these symptoms are rare.



(a)



(b)



This *Loa loa* worm, measuring about 55 mm long, was extracted from the conjunctiva of a patient with loiasis. The *Loa loa* has a complex

life cycle. Biting deerflies native to the rain forests of Central and West Africa transmit the larvae between humans. (credit a: modification of work by Eballe AO, Epée E, Koki G, Owono D, Mvogo CE, Bella AL; credit b: modification of work by NIAID; credit c: modification of work by Centers for Disease Control and Prevention)

Note:

- Describe the most common way to diagnose loiasis.

Note:

See a [video](#) of a live *Loa loa* microfilaria under the microscope.

Note:

Parasitic Skin and Eye Infections

The protozoan *Acanthamoeba* and the helminth *Loa loa* are two parasites capable of causing infections of the skin and eyes. [\[link\]](#) summarizes the characteristics of some common fungal infections of the skin.

Parasitic Skin and Eye Infections				
Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
<i>Acanthamoeba</i> keratitis	<i>Acanthamoeba</i>	Inflammation and damage to cornea; vision impairment or blindness	Exposure to pathogens in contaminated water or on contact lenses	Polyhexamethylene biguanide, chlorhexidine, azoles
Loiasis	<i>Loa loa</i>	Recurring fever and localized Calabar swelling, itching, and skin or eye pain during subcutaneous migration of worms	Larvae transmitted between humans by deerfly vector	Diethylcarbamazine, albendazole

Key Concepts and Summary

- The protozoan *Acanthamoeba* and the helminth *Loa loa* are two parasites that can breach the skin barrier, causing infections of the skin and eyes.
- ***Acanthamoeba keratitis*** is a parasitic infection of the eye that often results from improper disinfection of contact lenses or swimming while wearing contact lenses.
- **Loiasis**, or eye worm, is a disease endemic to Africa that is caused by parasitic worms that infect the subcutaneous tissue of the skin and eyes. It is transmitted by deerfly vectors.

Multiple Choice

Exercise:

Problem:

Which of the following is most likely to cause an *Acanthamoeba* infection?

- a. swimming in a lake while wearing contact lenses
- b. being bitten by deerflies in Central Africa
- c. living environments in a college dormitory with communal showers
- d. participating in a contact sport such as wrestling

Solution:

A

Exercise:

Problem: The parasitic *Loa loa* worm can cause great pain when it:

- a. moves through the bloodstream
- b. exits through the skin of the foot

- c. travels through the conjunctiva
 - d. enters the digestive tract
-

Solution:

C

Exercise:

Problem:

A patient tests positive for *Loa loa* antibodies. What does this test indicate?

- a. The individual was exposed to *Loa loa* at some point.
 - b. The individual is currently suffering from loiasis.
 - c. The individual has never been exposed to *Loa loa*.
 - d. The individual is immunosuppressed.
-

Solution:

A

Exercise:

Problem:

_____ is commonly treated with a combination of chlorhexidine and polyhexamethylene biguanide.

- a. *Acanthamoeba* keratitis
 - b. Sporotrichosis
 - c. Candidiasis
 - d. Loiasis
-

Solution:

A

Fill in the Blank

Exercise:

Problem: Eye worm is another name for _____.

Solution:

loiasis

Exercise:

Problem:

The _____ is the part of the eye that is damaged due to *Acanthamoeba* keratitis.

Solution:

cornea

Critical Thinking

Exercise:

Problem:

Why might a traveler to a region with *Loa loa* worm have a greater risk of serious infection compared with people who live in the region?

Exercise:

Problem:

What preventative actions might you recommend to a patient traveling to a region where loiasis is endemic?

Introduction

class="introduction"

Aerosols produced by sneezing, coughing, or even just speaking are an important mechanism for respiratory pathogen transmission. Simple actions, like covering your mouth when coughing or sneezing, can reduce the spread of these microbes.

(credit: modification of work by Centers for Disease Control and Prevention)



The respiratory tract is one of the main portals of entry into the human body for microbial pathogens. On average, a human takes about 20,000 breaths each day. This roughly corresponds to 10,000 liters, or 10 cubic meters, of air. Suspended within this volume of air are millions of microbes of terrestrial, animal, and human origin—including many potential pathogens. A few of these pathogens will cause relatively mild infections like sore throats and colds. Others, however, are less benign. According to the World Health Organization, respiratory tract infections such as tuberculosis, influenza, and pneumonia were responsible for more than 4 million deaths worldwide in 2012.[\[footnote\]](#)

World Health Organization. “The Top Ten Causes of Death.” May 2014.
<http://www.who.int/mediacentre/factsheets/fs310/en/>

At one time, it was thought that antimicrobial drugs and preventive vaccines might hold respiratory infections in check in the developed world, but recent developments suggest otherwise. The rise of multiple-antibiotic resistance in organisms like *Mycobacterium tuberculosis* has rendered many of our modern drugs ineffective. In addition, there has been a recent resurgence in diseases like whooping cough and measles, once-common childhood illnesses made rare by effective vaccines. Despite advances in medicine and public health programs, it is likely that respiratory pathogens will remain formidable adversaries for the foreseeable future.

Anatomy and Normal Microbiota of the Respiratory Tract

LEARNING OBJECTIVES

- Describe the major anatomical features of the upper and lower respiratory tract
- Describe the normal microbiota of the upper and lower respiratory tracts
- Explain how microorganisms overcome defenses of upper and lower respiratory-tract membranes to cause infection
- Explain how microbes and the respiratory system interact and modify each other in healthy individuals and during an infection

Note:

Part 1

John, a 65-year-old man with asthma and type 2 diabetes, works as a sales associate at a local home improvement store. Recently, he began to feel quite ill and made an appointment with his family physician. At the clinic, John reported experiencing headache, chest pain, coughing, and shortness of breath. Over the past day, he had also experienced some nausea and diarrhea. A nurse took his temperature and found that he was running a fever of 40 °C (104 °F).

John suggested that he must have a case of influenza (flu), and regretted that he had put off getting his flu vaccine this year. After listening to John's breathing through a stethoscope, the physician ordered a chest radiography and collected blood, urine, and sputum samples.

- Based on this information, what factors may have contributed to John's illness?

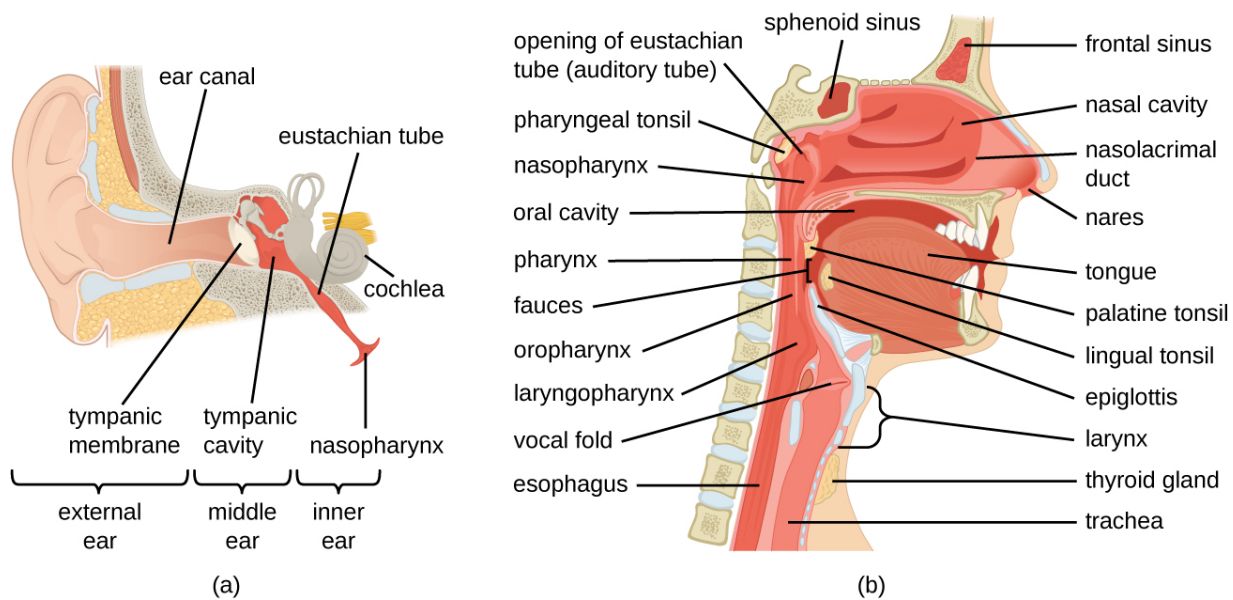
Jump to the [next](#) Clinical Focus box.

The primary function of the respiratory tract is to exchange gases (oxygen and carbon dioxide) for metabolism. However, inhalation and exhalation (particularly when forceful) can also serve as a vehicle of transmission for pathogens between individuals.

Anatomy of the Upper Respiratory System

The respiratory system can be conceptually divided into upper and lower regions at the point of the **epiglottis**, the structure that seals off the lower respiratory system from the **pharynx** during swallowing ([\[link\]](#)). The upper respiratory system is in direct contact with the external environment. The nares (or nostrils) are the external openings of the nose that lead back into the **nasal cavity**, a large air-filled space behind the nares. These anatomical sites constitute the primary opening and first section of the respiratory tract, respectively. The nasal cavity is lined with hairs that trap large particles, like dust and pollen, and prevent their access to deeper tissues. The nasal cavity is also lined with a mucous membrane and Bowman's glands that produce mucus to help trap particles and microorganisms for removal. The nasal cavity is connected to several other air-filled spaces. The sinuses, a set of four, paired small cavities in the skull, communicate with the nasal cavity through a series of small openings. The **nasopharynx** is part of the upper throat extending from the posterior nasal cavity. The nasopharynx carries air inhaled through the nose. The middle ear is connected to the nasopharynx through the **eustachian tube**. The middle ear is separated from the outer ear by the **tympanic membrane**, or ear drum. And finally, the lacrimal glands drain to the nasal cavity through the **nasolacrimal ducts** (tear ducts). The open connections between these sites allow microorganisms to move from the nasal cavity to the sinuses, middle ears (and back), and down into the lower respiratory tract from the nasopharynx.

The oral cavity is a secondary opening for the respiratory tract. The oral and nasal cavities connect through the fauces to the pharynx, or throat. The pharynx can be divided into three regions: the nasopharynx, the **oropharynx**, and the **laryngopharynx**. Air inhaled through the mouth does not pass through the nasopharynx; it proceeds first through the oropharynx and then through the laryngopharynx. The **palatine tonsils**, which consist of lymphoid tissue, are located within the oropharynx. The laryngopharynx, the last portion of the pharynx, connects to the **larynx**, which contains the vocal fold ([link](#)).



(a) The ear is connected to the upper respiratory tract by the eustachian tube, which opens to the nasopharynx. (b) The structures of the upper respiratory tract.

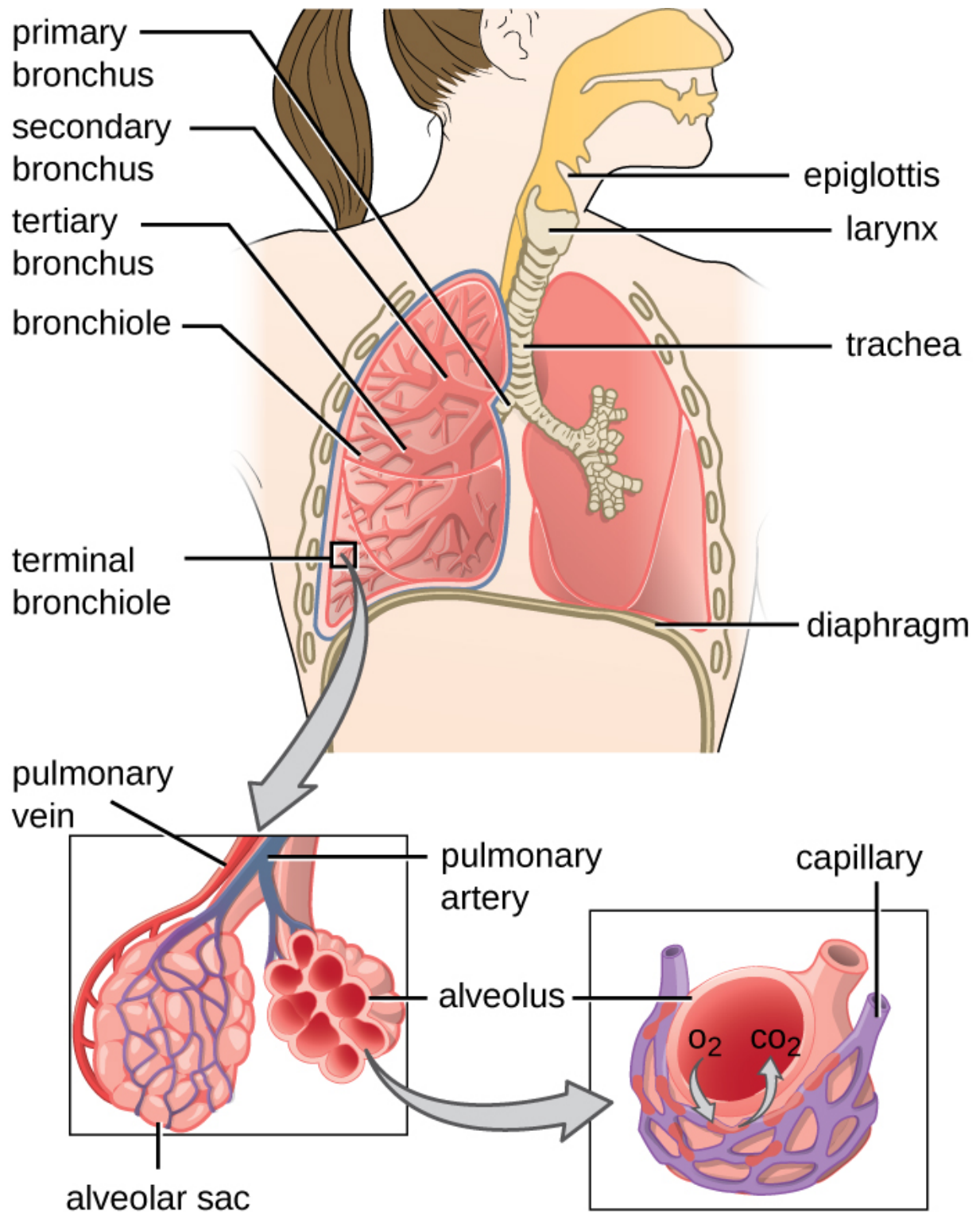
Note:

- Identify the sequence of anatomical structures through which microbes would pass on their way from the nares to the larynx.

- What two anatomical points do the eustachian tubes connect?

Anatomy of the Lower Respiratory System

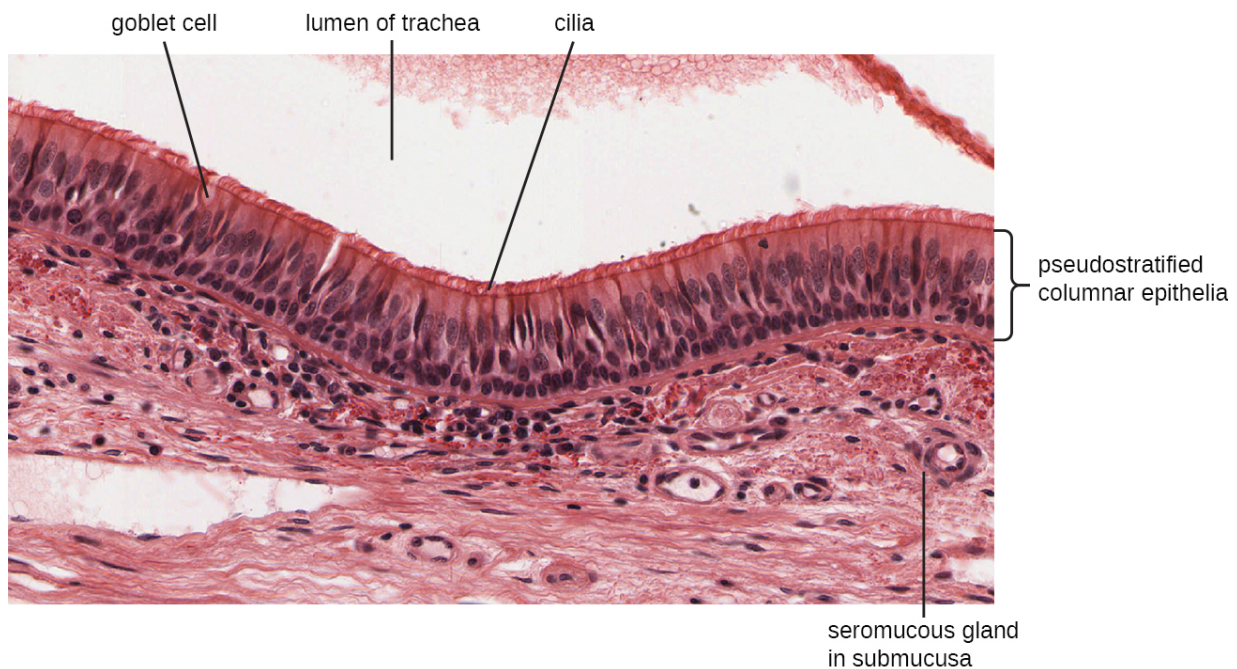
The lower respiratory system begins below the epiglottis in the larynx or voice box ([\[link\]](#)). The **trachea**, or windpipe, is a cartilaginous tube extending from the larynx that provides an unobstructed path for air to reach the lungs. The trachea bifurcates into the left and right **bronchi** as it reaches the lungs. These paths branch repeatedly to form smaller and more extensive networks of tubes, the **bronchioles**. The terminal bronchioles formed in this tree-like network end in cul-de-sacs called the **alveoli**. These structures are surrounded by capillary networks and are the site of gas exchange in the respiratory system. Human lungs contain on the order of 400,000,000 alveoli. The outer surface of the lungs is protected with a double-layered pleural membrane. This structure protects the lungs and provides lubrication to permit the lungs to move easily during respiration.



The structures of the lower respiratory tract are identified in this illustration. (credit: modification of work by National Cancer Institute)

Defenses of the Respiratory System

The inner lining of the respiratory system consists of mucous membranes ([link](#)) and is protected by multiple immune defenses. The goblet cells within the respiratory epithelium secrete a layer of sticky mucus. The viscosity and acidity of this secretion inhibits microbial attachment to the underlying cells. In addition, the respiratory tract contains ciliated epithelial cells. The beating cilia dislodge and propel the mucus, and any trapped microbes, upward to the epiglottis, where they will be swallowed. Elimination of microbes in this manner is referred to as the mucociliary escalator effect and is an important mechanism that prevents inhaled microorganisms from migrating further into the lower respiratory tract.



This micrograph shows the structure of the mucous membrane of the respiratory tract. (credit: modification of micrograph provided by the Regents of University of Michigan Medical School © 2012)

The upper respiratory system is under constant surveillance by mucosa-associated lymphoid tissue (MALT), including the adenoids and tonsils. Other mucosal defenses include secreted antibodies (IgA), lysozyme, surfactant, and antimicrobial peptides called defensins. Meanwhile, the lower respiratory tract is protected by alveolar macrophages. These phagocytes efficiently kill any microbes that manage to evade the other defenses. The combined action of these factors renders the lower respiratory tract nearly devoid of colonized microbes.

Note:

- Identify the sequence of anatomical structures through which microbes would pass on their way from the larynx to the alveoli.
- Name some defenses of the respiratory system that protect against microbial infection.

Normal Microbiota of the Respiratory System

The upper respiratory tract contains an abundant and diverse microbiota. The nasal passages and sinuses are primarily colonized by members of the Firmicutes, Actinobacteria, and Proteobacteria. The most common bacteria identified include *Staphylococcus epidermidis*, viridans group streptococci (VGS), *Corynebacterium* spp. (diphtheroids), *Propionibacterium* spp., and *Haemophilus* spp. The oropharynx includes many of the same isolates as the nose and sinuses, with the addition of variable numbers of bacteria like species of *Prevotella*, *Fusobacterium*, *Moraxella*, and *Eikenella*, as well as some *Candida* fungal isolates. In addition, many healthy humans asymptotically carry potential pathogens in the upper respiratory tract. As much as 20% of the population carry *Staphylococcus aureus* in their nostrils.[\[footnote\]](#) The pharynx, too, can be colonized with pathogenic strains of *Streptococcus*, *Haemophilus*, and *Neisseria*.

J. Kluytmans et al. “Nasal Carriage of *Staphylococcus aureus*: Epidemiology, Underlying Mechanisms, and Associated Risks.” *Clinical*

Microbiology Reviews 10 no. 3 (1997):505–520.

The lower respiratory tract, by contrast, is scantily populated with microbes. Of the organisms identified in the lower respiratory tract, species of *Pseudomonas*, *Streptococcus*, *Prevotella*, *Fusobacterium*, and *Veillonella* are the most common. It is not clear at this time if these small populations of bacteria constitute a normal microbiota or if they are transients.

Many members of the respiratory system's normal microbiota are opportunistic pathogens. To proliferate and cause host damage, they first must overcome the immune defenses of respiratory tissues. Many mucosal pathogens produce virulence factors such as adhesins that mediate attachment to host epithelial cells, or polysaccharide capsules that allow microbes to evade phagocytosis. The endotoxins of gram-negative bacteria can stimulate a strong inflammatory response that damages respiratory cells. Other pathogens produce exotoxins, and still others have the ability to survive within the host cells. Once an infection of the respiratory tract is established, it tends to impair the mucociliary escalator, limiting the body's ability to expel the invading microbes, thus making it easier for pathogens to multiply and spread.

Vaccines have been developed for many of the most serious bacterial and viral pathogens. Several of the most important respiratory pathogens and their vaccines, if available, are summarized in [\[link\]](#). Components of these vaccines will be explained later in the chapter.

Some Important Respiratory Diseases and Vaccines

Disease	Pathogen	Available Vaccine(s) [footnote] Full names of vaccines listed in table: <i>Haemophilus influenzae</i> type B (Hib); Diphtheria, tetanus, and acellular pertussis (DtaP); tetanus, diphtheria, and acellular pertussis (Tdap); diphtheria and tetanus (DT); tetanus and diphtheria (Td); diphtheria, pertussis, and tetanus (DTP); Bacillus Calmette-Guérin; Measles, mumps, rubella (MMR)
Chickenpox/shingles	Varicella-zoster virus	Varicella (chickenpox) vaccine, herpes zoster (shingles) vaccine
Common cold	Rhinovirus	None
Diphtheria	<i>Corynebacterium diphtheriae</i>	DtaP, Tdap, DT, Td, DTP

Epiglottitis, otitis media	<i>Haemophilus influenzae</i>	Hib
Influenza	Influenza viruses	Inactivated, FluMist
Measles	Measles virus	MMR
Pertussis	<i>Bordetella pertussis</i>	DTaP, Tdap
Pneumonia	<i>Streptococcus pneumoniae</i>	Pneumococcal conjugate vaccine (PCV13), pneumococcal polysaccharide vaccine (PPSV23)
Rubella (German measles)	Rubella virus	MMR
Severe acute respiratory syndrome (SARS)	SARS-associated coronavirus (SARS-CoV)	None
Tuberculosis	<i>Mycobacterium tuberculosis</i>	BCG

Note:

- What are some pathogenic bacteria that are part of the normal microbiota of the respiratory tract?
- What virulence factors are used by pathogens to overcome the immune protection of the respiratory tract?

Signs and Symptoms of Respiratory Infection

Microbial diseases of the respiratory system typically result in an acute inflammatory response. These infections can be grouped by the location affected and have names ending in “itis”, which literally means *inflammation of*. For instance, **rhinitis** is an inflammation of the nasal cavities, often characteristic of the common cold. Rhinitis may also be associated with hay fever allergies or other irritants. Inflammation of the sinuses is called **sinusitis** inflammation of the ear is called **otitis**. Otitis media is an inflammation of the middle ear. A variety of microbes can cause **pharyngitis**, commonly known as a sore throat. An inflammation of the larynx is called **laryngitis**. The resulting inflammation may interfere with vocal cord function, causing voice loss. When tonsils are inflamed, it is called **tonsillitis**. Chronic cases of tonsillitis may be treated surgically with tonsillectomy. More rarely, the epiglottis can be infected, a condition called **epiglottitis**. In the lower respiratory system, the inflammation of the bronchial tubes results in **bronchitis**. Most serious of all is **pneumonia**, in which the alveoli in the lungs are infected and become inflamed. Pus and edema accumulate and fill the alveoli with fluids (called consolidations). This reduces the lungs’ ability to exchange gases and often results in a productive cough expelling phlegm and mucus. Cases of pneumonia can range from mild to life-threatening, and remain an important cause of mortality in the very young and very old.

Note:

- Describe the typical symptoms of rhinitis, sinusitis, pharyngitis, and laryngitis.

Note:

Smoking-Associated Pneumonia

Camila is a 22-year-old student who has been a chronic smoker for 5 years. Recently, she developed a persistent cough that has not responded to over-

the-counter treatments. Her doctor ordered a chest radiograph to investigate. The radiological results were consistent with pneumonia. In addition, *Streptococcus pneumoniae* was isolated from Camila's sputum. Smokers are at a greater risk of developing pneumonia than the general population. Several components of tobacco smoke have been demonstrated to impair the lungs' immune defenses. These effects include disrupting the function of the ciliated epithelial cells, inhibiting phagocytosis, and blocking the action of antimicrobial peptides. Together, these lead to a dysfunction of the mucociliary escalator effect. The organisms trapped in the mucus are therefore able to colonize the lungs and cause infections rather than being expelled or swallowed.

Key Concepts and Summary

- The respiratory tract is divided into upper and lower regions at the **epiglottis**.
- Air enters the upper respiratory tract through the **nasal cavity** and mouth, which both lead to the **pharynx**. The lower respiratory tract extends from the **larynx** into the **trachea** before branching into the **bronchi**, which divide further to form the **bronchioles**, which terminate in **alveoli**, where gas exchange occurs.
- The upper respiratory tract is colonized by an extensive and diverse normal microbiota, many of which are potential pathogens. Few microbial inhabitants have been found in the lower respiratory tract, and these may be transients.
- Members of the normal microbiota may cause opportunistic infections, using a variety of strategies to overcome the innate nonspecific defenses (including the mucociliary escalator) and adaptive specific defenses of the respiratory system.
- Effective vaccines are available for many common respiratory pathogens, both bacterial and viral.
- Most respiratory infections result in inflammation of the infected tissues; these conditions are given names ending in *-itis*, such as **rhinitis**, **sinusitis**, **otitis**, **pharyngitis**, and **bronchitis**.

Multiple Choice

Exercise:

Problem:

Which of the following is not directly connected to the nasopharynx?

- A. middle ear
- B. oropharynx
- C. lacrimal glands
- D. nasal cavity

Solution:

C

Exercise:

Problem:

What type of cells produce the mucus for the mucous membranes?

- A. goblet cells
- B. macrophages
- C. phagocytes
- D. ciliated epithelial cells

Solution:

A

Exercise:

Problem:

Which of these correctly orders the structures through which air passes during inhalation?

- A. pharynx → trachea → larynx → bronchi
 - B. pharynx → larynx → trachea → bronchi
 - C. larynx → pharynx → bronchi → trachea
 - D. larynx → pharynx → trachea → bronchi
-

Solution:

B

Exercise:

Problem:

The _____ separates the upper and lower respiratory tract.

- A. bronchi
 - B. larynx
 - C. epiglottis
 - D. palatine tonsil
-

Solution:

C

Exercise:

Problem:

Which microbial virulence factor is most important for attachment to host respiratory tissues?

- A. adhesins
 - B. lipopolysaccharide
 - C. hyaluronidase
 - D. capsules
-

Solution:

A

Fill in the Blank

Exercise:

Problem:

Unattached microbes are moved from the lungs to the epiglottis by the _____ effect.

Solution:

mucociliary escalator

Exercise:

Problem:

Many bacterial pathogens produce _____ to evade phagocytosis.

Solution:

capsules

Exercise:

Problem:

The main type of antibody in the mucous membrane defenses is _____.

Solution:

IgA

Exercise:

Problem: _____ results from an inflammation of the “voice box.”

Solution:

Laryngitis

Exercise:

Problem: _____ phagocytize potential pathogens in the lower lung.

Solution:

Alveolar macrophages

Short Answer**Exercise:**

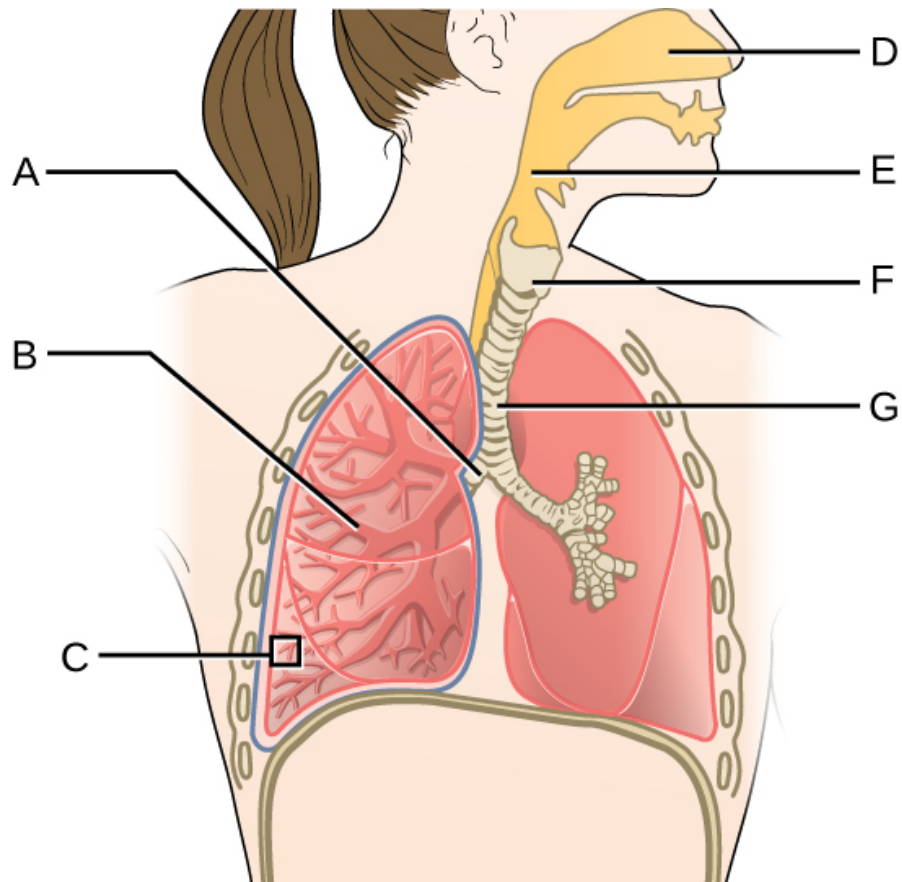
Problem: Explain why the lower respiratory tract is essentially sterile.

Exercise:

Problem: Explain why pneumonia is often a life-threatening disease.

Critical Thinking**Exercise:****Problem:**

Name each of the structures of the respiratory tract shown, and state whether each has a relatively large or small normal microbiota.



(credit: modification of work by National Cancer Institute)

Exercise:

Problem:

Cystic fibrosis causes, among other things, excess mucus to be formed in the lungs. The mucus is very dry and caked, unlike the moist, more-fluid mucus of normal lungs. What effect do you think that has on the lung's defenses?

Exercise:

Problem:

Why do you think smokers are more likely to suffer from respiratory tract infections?

Bacterial Infections of the Respiratory Tract

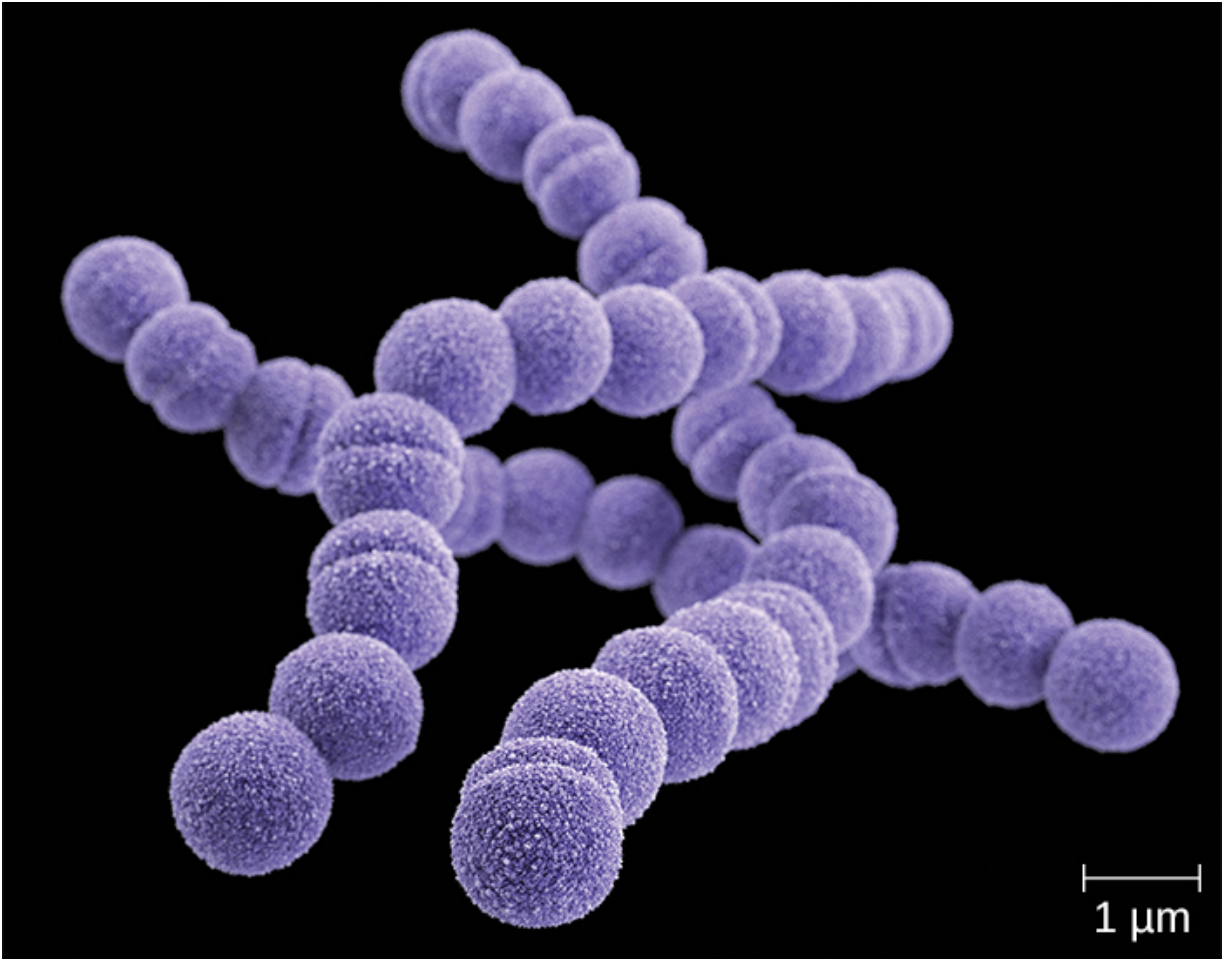
LEARNING OBJECTIVES

- Identify the most common bacteria that can cause infections of the upper and lower respiratory tract
- Compare the major characteristics of specific bacterial diseases of the respiratory tract

The respiratory tract can be infected by a variety of bacteria, both gram positive and gram negative. Although the diseases that they cause may range from mild to severe, in most cases, the microbes remain localized within the respiratory system. Fortunately, most of these infections also respond well to antibiotic therapy.

Streptococcal Infections

A common upper respiratory infection, **streptococcal pharyngitis (strep throat)** is caused by *Streptococcus pyogenes*. This gram-positive bacterium appears as chains of cocci, as seen in [\[link\]](#). Rebecca Lancefield serologically classified streptococci in the 1930s using carbohydrate antigens from the bacterial cell walls. *S. pyogenes* is the sole member of the Lancefield group A streptococci and is often referred to as GAS, or group A strep.



This scanning electron micrograph of *Streptococcus pyogenes* shows the characteristic cellular phenotype resembling chains of cocci. (credit: modification of work by U.S. Centers for Disease Control and Prevention - Medical Illustrator)

Similar to streptococcal infections of the skin, the mucosal membranes of the pharynx are damaged by the release of a variety of exoenzymes and exotoxins by this extracellular pathogen. Many strains of *S. pyogenes* can degrade connective tissues by using hyaluronidase, collagenase and streptokinase. Streptokinase activates plasmin, which leads to degradation of fibrin and, in turn, dissolution of blood clots, which assists in the spread of the pathogen. Released toxins include streptolysins that can destroy red and white blood cells. The classic signs of streptococcal pharyngitis are a

fever higher than 38 °C (100.4 °F); intense pharyngeal pain; erythema associated with pharyngeal inflammation; and swollen, dark-red palatine tonsils, often dotted with patches of pus; and petechiae (microcapillary hemorrhages) on the soft or hard palate (roof of the mouth) ([\[link\]](#)). The submandibular lymph nodes beneath the angle of the jaw are also often swollen during strep throat.

Some strains of group A streptococci produce **erythrogenic toxin**. This exotoxin is encoded by a temperate bacteriophage (bacterial virus) and is an example of phage conversion (see [The Viral Life Cycle](#)). The toxin attacks the plasma membranes of capillary endothelial cells and leads to **scarlet fever** (or scarlatina), a disseminated fine red rash on the skin, and strawberry tongue, a red rash on the tongue ([\[link\]](#)). Severe cases may even lead to streptococcal toxic shock syndrome (STSS), which results from massive superantigen production that leads to septic shock and death.

S. pyogenes can be easily spread by direct contact or droplet transmission through coughing and sneezing. The disease can be diagnosed quickly using a rapid enzyme immunoassay for the group A antigen. However, due to a significant rate of false-negative results (up to 30%[\[footnote\]](#)), culture identification is still the gold standard to confirm pharyngitis due to *S. pyogenes*. *S. pyogenes* can be identified as a catalase-negative, beta hemolytic bacterium that is susceptible to 0.04 units of bacitracin. Antibiotic resistance is limited for this bacterium, so most β -lactams remain effective; oral amoxicillin and intramuscular penicillin G are those most commonly prescribed.

WL Lean et al. “Rapid Diagnostic Tests for Group A Streptococcal Pharyngitis: A Meta-Analysis.” *Pediatrics* 134, no. 4 (2014):771–781.



(a)



(b)

Streptococcal infections of the respiratory tract may cause localized pharyngitis or systemic signs and symptoms. (a) The characteristic appearance of strep throat: bright red arches of inflammation with the presence of dark-red spots (petechiae). (b) Scarlet fever presents as a rash on the skin. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Alicia Williams)

Sequelae of *S. pyogenes* Infections

One reason strep throat infections are aggressively treated with antibiotics is because they can lead to serious **sequelae**, later clinical consequences of a primary infection. It is estimated that 1%–3% of untreated *S. pyogenes* infections can be followed by nonsuppurative (without the production of pus) sequelae that develop 1–3 weeks after the acute infection has resolved. Two such sequelae are **acute rheumatic fever** and **acute glomerulonephritis**.

Acute rheumatic fever can follow pharyngitis caused by specific rheumatogenic strains of *S. pyogenes* (strains 1, 3, 5, 6, and 18). Although

the exact mechanism responsible for this sequela remains unclear, molecular mimicry between the M protein of rheumatogenic strains of *S. pyogenes* and heart tissue is thought to initiate the autoimmune attack. The most serious and lethal clinical manifestation of rheumatic fever is damage to and inflammation of the heart (carditis). Acute glomerulonephritis also results from an immune response to streptococcal antigens following pharyngitis and cutaneous infections. Acute glomerulonephritis develops within 6–10 days after pharyngitis, but can take up to 21 days after a cutaneous infection. Similar to acute rheumatic fever, there are strong associations between specific nephritogenic strains of *S. pyogenes* and acute glomerulonephritis, and evidence suggests a role for antigen mimicry and autoimmunity. However, the primary mechanism of acute glomerulonephritis appears to be the formation of immune complexes between *S. pyogenes* antigens and antibodies, and their deposition between endothelial cells of the glomeruli of kidney. Inflammatory response against the immune complexes leads to damage and inflammation of the glomeruli (glomerulonephritis).

Note:

- What are the symptoms of strep throat?
- What is erythrogenic toxin and what effect does it have?
- What are the causes of rheumatic fever and acute glomerulonephritis?

Acute Otitis Media

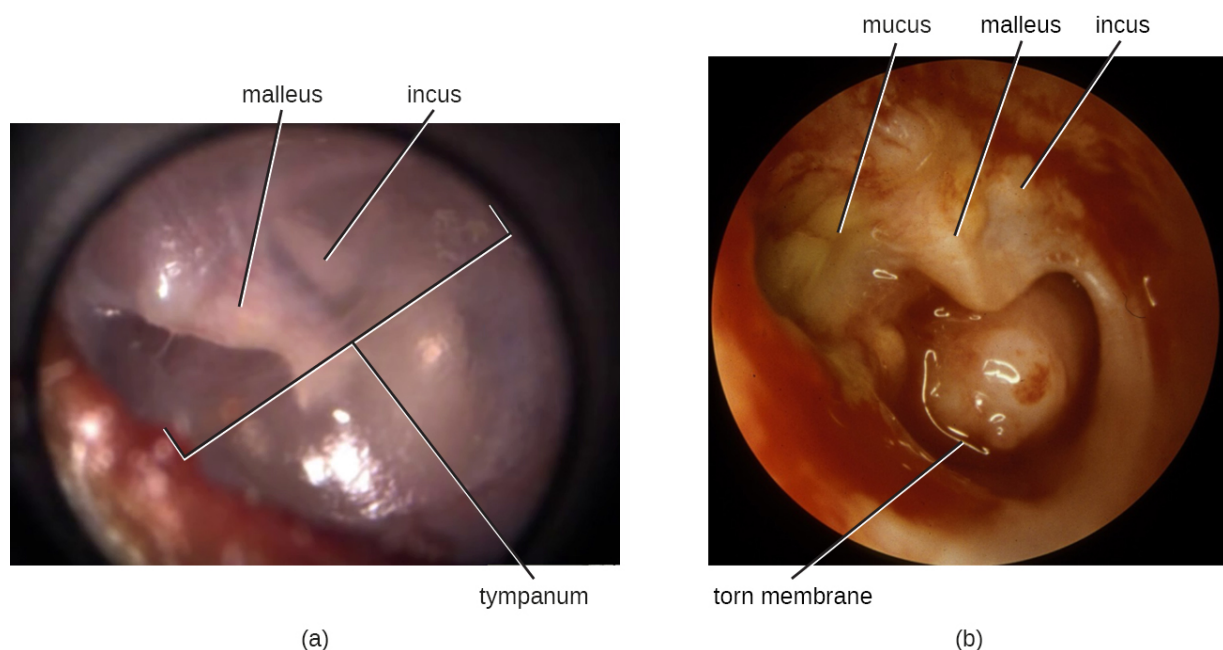
An infection of the middle ear is called **acute otitis media (AOM)**, but often it is simply referred to as an earache. The condition is most common between ages 3 months and 3 years. In the United States, AOM is the second-leading cause of visits to pediatricians by children younger than age 5 years, and it is the leading indication for antibiotic prescription. [\[footnote\]](#) G. Worrall. “Acute Otitis Media.” *Canadian Family Physician* 53 no. 12 (2007):2147–2148.

AOM is characterized by the formation and accumulation of pus in the middle ear. Unable to drain, the pus builds up, resulting in moderate to severe bulging of the tympanic membrane and otalgia (ear pain). Inflammation resulting from the infection leads to swelling of the eustachian tubes, and may also lead to fever, nausea, vomiting, and diarrhea, particularly in infants. Infants and toddlers who cannot yet speak may exhibit nonverbal signs suggesting AOM, such as holding, tugging, or rubbing of the ear, as well as uncharacteristic crying or distress in response to the pain.

AOM can be caused by a variety of bacteria. Among neonates, *S. pneumoniae* is the most common cause of AOM, but *Escherichia coli*, *Enterococcus* spp., and group B *Streptococcus* species can also be involved. In older infants and children younger than 14 years old, the most common bacterial causes are *S. pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis*. Among *S. pneumoniae* infections, encapsulated strains are frequent causes of AOM. By contrast, the strains of *H. influenzae* and *M. catarrhalis* that are responsible for AOM do not possess a capsule. Rather than direct tissue damage by these pathogens, bacterial components such as lipopolysaccharide (LPS) in gram-negative pathogens induce an inflammatory response that causes swelling, pus, and tissue damage within the middle ear ([\[link\]](#)).

Any blockage of the eustachian tubes, with or without infection, can cause fluid to become trapped and accumulate in the middle ear. This is referred to as **otitis media with effusion (OME)**. The accumulated fluid offers an excellent reservoir for microbial growth and, consequently, secondary bacterial infections often ensue. This can lead to recurring and chronic earaches, which are especially common in young children. The higher incidence in children can be attributed to many factors. Children have more upper respiratory infections, in general, and their eustachian tubes are also shorter and drain at a shallower angle. Young children also tend to spend more time lying down than adults, which facilitates drainage from the nasopharynx through the eustachian tube and into the middle ear. Bottle feeding while lying down enhances this risk because the sucking action on the bottle causes negative pressure to build up within the eustachian tube, promoting the movement of fluid and bacteria from the nasopharynx.

Diagnosis is typically made based on clinical signs and symptoms, without laboratory testing to determine the specific causative agent. Antibiotics are frequently prescribed for the treatment of AOM. High-dose amoxicillin is the first-line drug, but with increasing resistance concerns, macrolides and cephalosporins may also be used. The pneumococcal conjugate vaccine (PCV13) contains serotypes that are important causes of AOM, and vaccination has been shown to decrease the incidence of AOM. Vaccination against influenza has also been shown to decrease the risk for AOM, likely because viral infections like influenza predispose patients to secondary infections with *S. pneumoniae*. Although there is a conjugate vaccine available for the invasive serotype B of *H. influenzae*, this vaccine does not impact the incidence of *H. influenzae* AOM. Because unencapsulated strains of *H. influenzae* and *M. catarrhalis* are involved in AOM, vaccines against bacterial cellular factors other than capsules will need to be developed.



(a) A healthy tympanic membrane; the middle ear bones can be seen behind the membrane. (b) An ear with chronic inflammation that has resulted in a torn membrane, erosion of the inner ear bones, and mucus

buildup. (credit a: modification of work by “DrER.tv”/YouTube; credit b: modification of work by Li Mg, Hotez PJ, Vrabec JT, Donovan DT)

Bacterial Rhinosinusitis

The microbial community of the nasopharynx is extremely diverse and harbors many opportunistic pathogens, so it is perhaps not surprising that infections leading to rhinitis and sinusitis have many possible causes. These conditions often occur as secondary infections after a viral infection, which effectively compromises the immune defenses and allows the opportunistic bacteria to establish themselves. Bacterial sinusitis involves infection and inflammation within the paranasal sinuses. Because bacterial sinusitis rarely occurs without rhinitis, the preferred term is rhinosinusitis. The most common causes of bacterial rhinosinusitis are similar to those for AOM, including *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*.

Note:

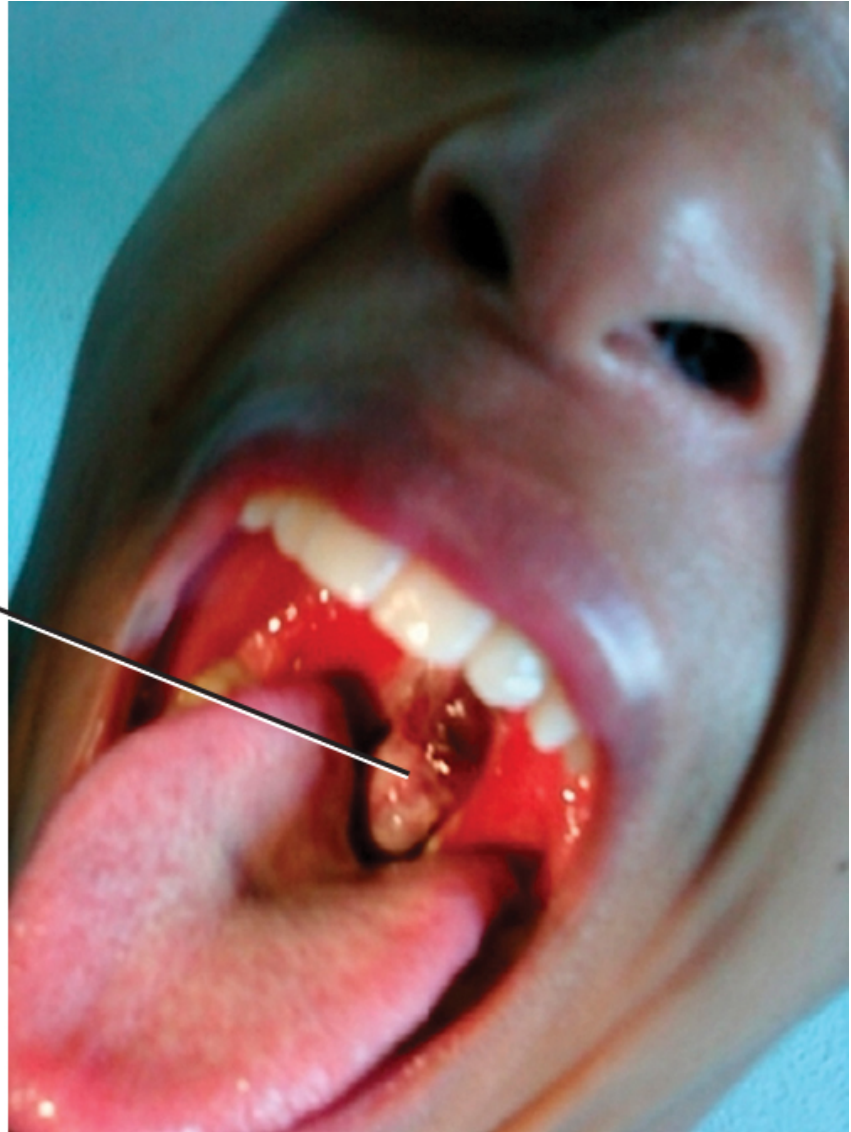
- What are the usual causative agents of acute otitis media?
- What factors facilitate acute otitis media with effusion in young children?
- What factor often triggers bacterial rhinosinusitis?

Diphtheria

The causative agent of **diphtheria**, *Corynebacterium diphtheriae*, is a club-shaped, gram-positive rod that belongs to the phylum Actinobacteria. Diphtheroids are common members of the normal nasopharyngeal microbiota. However, some strains of *C. diphtheriae* become pathogenic because of the presence of a temperate bacteriophage-encoded protein—the

diphtheria toxin. Diphtheria is typically a respiratory infection of the oropharynx but can also cause impetigo-like lesions on the skin. Although the disease can affect people of all ages, it tends to be most severe in those younger than 5 years or older than 40 years. Like strep throat, diphtheria is commonly transmitted in the droplets and aerosols produced by coughing. After colonizing the throat, the bacterium remains in the oral cavity and begins producing the diphtheria toxin. This protein is an A-B toxin that blocks host-cell protein synthesis by inactivating elongation factor (EF)-2 (see [Virulence Factors of Bacterial and Viral Pathogens](#)). The toxin's action leads to the death of the host cells and an inflammatory response. An accumulation of grayish exudate consisting of dead host cells, pus, red blood cells, fibrin, and infectious bacteria results in the formation of a **pseudomembrane**. The pseudomembrane can cover mucous membranes of the nasal cavity, tonsils, pharynx, and larynx ([link](#)). This is a classic sign of diphtheria. As the disease progresses, the pseudomembrane can enlarge to obstruct the fauces of the pharynx or trachea and can lead to suffocation and death. Sometimes, **intubation**, the placement of a breathing tube in the trachea, is required in advanced infections. If the diphtheria toxin spreads throughout the body, it can damage other tissues as well. This can include myocarditis (heart damage) and nerve damage that may impair breathing.

pseudomembrane



The pseudomembrane in a patient with diphtheria presents as a leathery gray patch consisting of dead cells, pus, fibrin, red blood cells, and infectious microbes. (credit: modification of work by Putnong N, Agustin G, Pasubillo M, Miyagi K, Dimaano EM)

The presumptive diagnosis of diphtheria is primarily based on the clinical symptoms (i.e., the pseudomembrane) and vaccination history, and is typically confirmed by identifying bacterial cultures obtained from throat swabs. The diphtheria toxin itself can be directly detected in vitro using

polymerase chain reaction (PCR)-based, direct detection systems for the diphtheria *tox* gene, and immunological techniques like radial immunodiffusion or Elek's immunodiffusion test.

Broad-spectrum antibiotics like penicillin and erythromycin tend to effectively control *C. diphtheriae* infections. Regrettably, they have no effect against preformed toxins. If toxin production has already occurred in the patient, antitoxins (preformed antibodies against the toxin) are administered. Although this is effective in neutralizing the toxin, the antitoxins may lead to serum sickness because they are produced in horses (see [Hypersensitivities](#)).

Widespread vaccination efforts have reduced the occurrence of diphtheria worldwide. There are currently four combination toxoid vaccines available that provide protection against diphtheria and other diseases: DTaP, Tdap, DT, and Td. In all cases, the letters “d,” “t,” and “p” stand for diphtheria, tetanus, and pertussis, respectively; the “a” stands for acellular. If capitalized, the letters indicate a full-strength dose; lowercase letters indicate reduced dosages. According to current recommendations, children should receive five doses of the DTaP vaccine in their youth and a Td booster every 10 years. Children with adverse reactions to the pertussis vaccine may be given the DT vaccine in place of the DTaP.

Note:

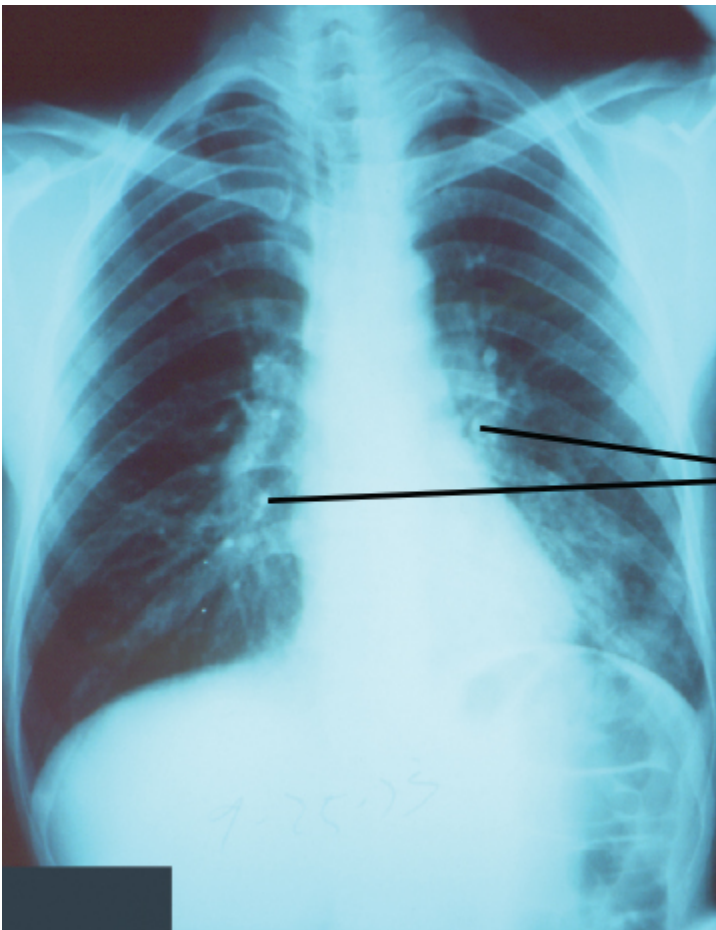
- What effect does diphtheria toxin have?
- What is the pseudomembrane composed of?

Bacterial Pneumonia

Pneumonia is a general term for infections of the lungs that lead to inflammation and accumulation of fluids and white blood cells in the alveoli. Pneumonia can be caused by bacteria, viruses, fungi, and other

organisms, although the vast majority of pneumonias are bacterial in origin. Bacterial pneumonia is a prevalent, potentially serious infection; it caused more 50,000 deaths in the United States in 2014.[\[footnote\]](#) As the alveoli fill with fluids and white blood cells (consolidation), air exchange becomes impaired and patients experience respiratory distress ([\[link\]](#)). In addition, pneumonia can lead to pleurisy, an infection of the pleural membrane surrounding the lungs, which can make breathing very painful. Although many different bacteria can cause pneumonia under the right circumstances, three bacterial species cause most clinical cases: *Streptococcus pneumoniae*, *H. influenzae*, and *Mycoplasma pneumoniae*. In addition to these, we will also examine some of the less common causes of pneumonia.

KD Kochanek et al. “Deaths: Final Data for 2014.” *National Vital Statistics Reports* 65 no 4 (2016).

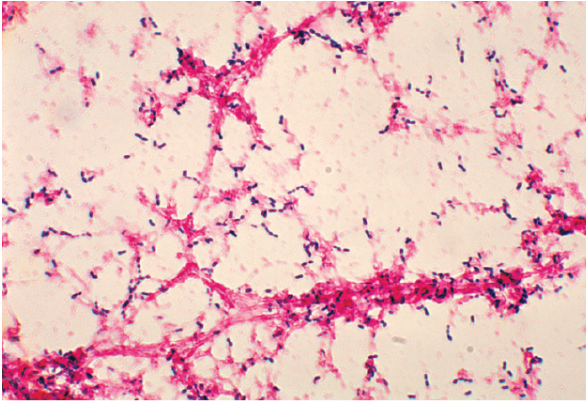


lesions

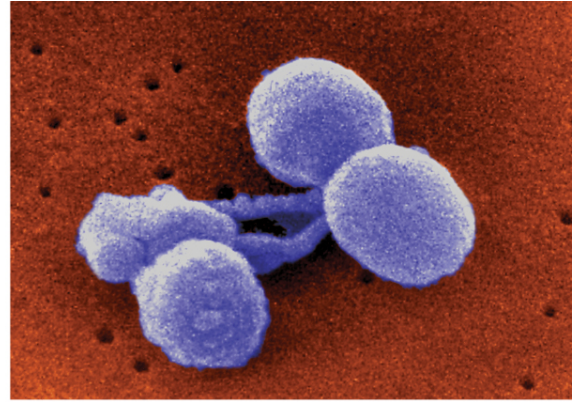
A chest radiograph of a patient with pneumonia shows the consolidations (lesions) present as opaque patches.
(credit: modification of work by Centers for Disease Control and Prevention)

Pneumococcal Pneumonia

The most common cause of community-acquired bacterial pneumonia is *Streptococcus pneumoniae*. This gram-positive, alpha hemolytic streptococcus is commonly found as part of the normal microbiota of the human respiratory tract. The cells tend to be somewhat lancet-shaped and typically appear as pairs ([link](#)). The pneumococci initially colonize the bronchioles of the lungs. Eventually, the infection spreads to the alveoli, where the microbe's polysaccharide capsule interferes with phagocytic clearance. Other virulence factors include autolysins like Lyt A, which degrade the microbial cell wall, resulting in cell lysis and the release of cytoplasmic virulence factors. One of these factors, pneumolysin O, is important in disease progression; this pore-forming protein damages host cells, promotes bacterial adherence, and enhances pro-inflammatory cytokine production. The resulting inflammatory response causes the alveoli to fill with exudate rich in neutrophils and red blood cells. As a consequence, infected individuals develop a productive cough with bloody sputum.



(a)



(b)

(a) This micrograph of *Streptococcus pneumoniae* grown from a blood culture shows the characteristic lancet-shaped diplococcal morphology. (b) A colorized scanning electron micrograph of *S. pneumoniae*. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Janice Carr, Centers for Disease Control and Prevention)

Pneumococci can be presumptively identified by their distinctive gram-positive, lancet-shaped cell morphology and diplococcal arrangement. In blood agar cultures, the organism demonstrates alpha hemolytic colonies that are autolytic after 24 to 48 hours. In addition, *S. pneumoniae* is extremely sensitive to optochin and colonies are rapidly destroyed by the addition of 10% solution of sodium deoxycholate. All clinical pneumococcal isolates are serotyped using the quellung reaction with typing antisera produced by the CDC. Positive quellung reactions are considered definitive identification of pneumococci.

Antibiotics remain the mainstay treatment for pneumococci. β -Lactams like penicillin are the first-line drugs, but resistance to β -lactams is a growing problem. When β -lactam resistance is a concern, macrolides and fluoroquinolones may be prescribed. However, *S. pneumoniae* resistance to macrolides and fluoroquinolones is increasing as well, limiting the therapeutic options for some infections. There are currently two pneumococcal vaccines available: pneumococcal conjugate vaccine

(PCV13) and pneumococcal polysaccharide vaccine (PPSV23). These are generally given to the most vulnerable populations of individuals: children younger than 2 years and adults older than 65 years.

***Haemophilus* Pneumonia**

Encapsulated strains of *Haemophilus influenzae* are known for causing meningitis, but nonencapsulated strains are important causes of pneumonia. This small, gram-negative coccobacillus is found in the pharynx of the majority of healthy children; however, *Haemophilus* pneumonia is primarily seen in the elderly. Like other pathogens that cause pneumonia, *H. influenzae* is spread by droplets and aerosols produced by coughing. A fastidious organism, *H. influenzae* will only grow on media with available factor X (hemin) and factor V (NAD), like chocolate agar ([\[link\]](#)). Serotyping must be performed to confirm identity of *H. influenzae* isolates.

Infections of the alveoli by *H. influenzae* result in inflammation and accumulation of fluids. Increasing resistance to β -lactams, macrolides, and tetracyclines presents challenges for the treatment of *Haemophilus* pneumonia. Resistance to the fluoroquinolones is rare among isolates of *H. influenzae* but has been observed. As discussed for AOM, a vaccine directed against nonencapsulated *H. influenzae*, if developed, would provide protection against pneumonia caused by this pathogen.



Culture of *Haemophilus influenzae* on a chocolate agar plate. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

Why Me?

Tracy is a 6-year old who developed a serious cough that would not seem to go away. After 2 weeks, her parents became concerned and took her to the pediatrician, who suspected a case of bacterial pneumonia. Tests confirmed that the cause was *Haemophilus influenzae*. Fortunately, Tracy responded well to antibiotic treatment and eventually made a full recovery.

Because there had been several other cases of bacterial pneumonia at Tracy's elementary school, local health officials urged parents to have their children screened. Of the children who were screened, it was discovered that greater than 50% carried *H. influenzae* in their nasal cavities, yet all but two of them were asymptomatic.

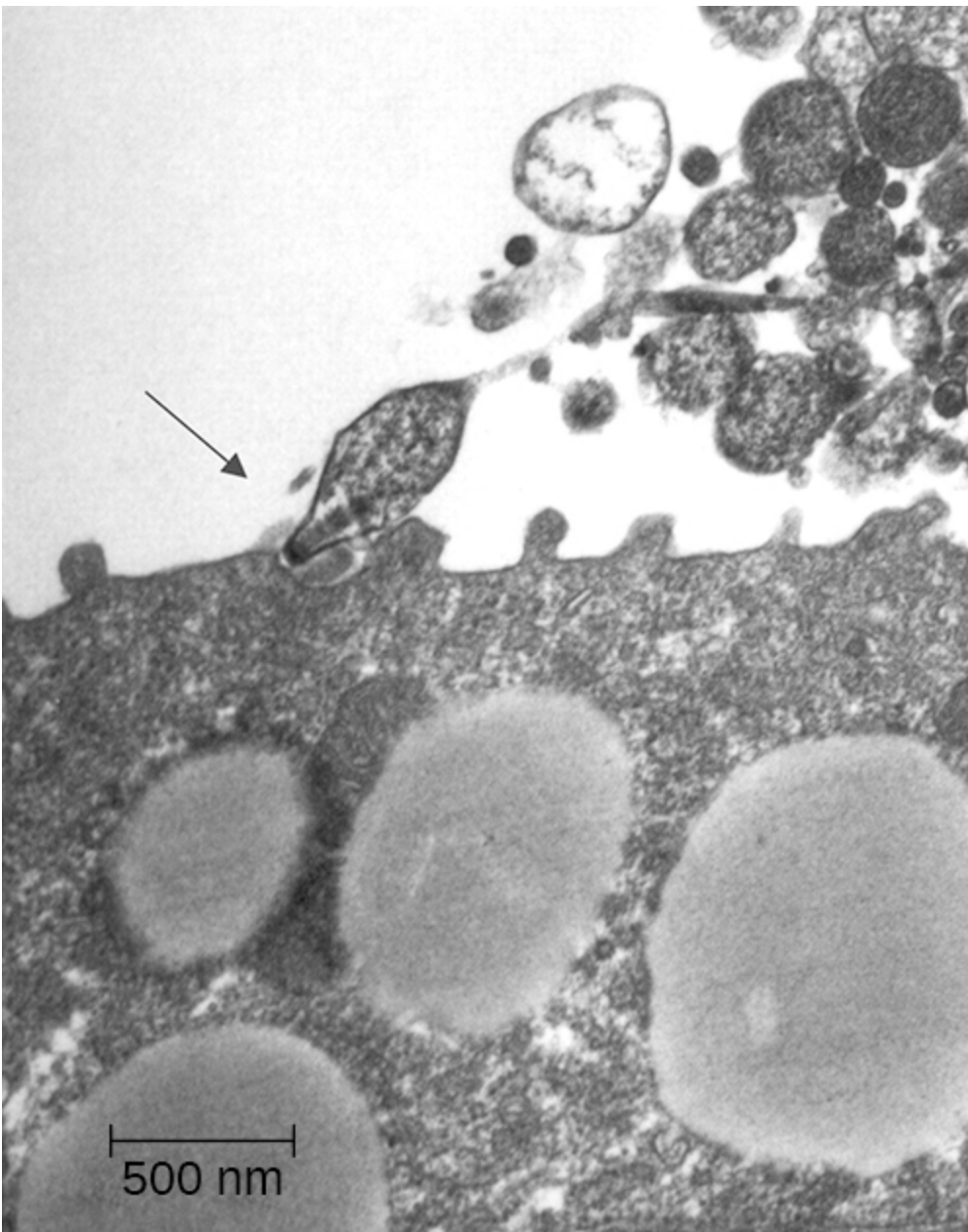
Why is it that some individuals become seriously ill from bacterial infections that seem to have little or no effect on others? The pathogenicity of an organism—its ability to cause host damage—is not solely a property of the microorganism. Rather, it is the product of a complex relationship between the microbe's virulence factors and the immune defenses of the individual. Preexisting conditions and environmental factors such as exposure to secondhand smoke can make some individuals more susceptible to infection by producing conditions favorable to microbial growth or compromising the immune system. In addition, individuals may have genetically determined immune factors that protect them—or not—from particular strains of pathogens. The interactions between these host factors and the pathogenicity factors produced by the microorganism ultimately determine the outcome of the infection. A clearer understanding of these interactions may allow for better identification of at-risk individuals and prophylactic interventions in the future.

***Mycoplasma Pneumonia* (Walking Pneumonia)**

Primary atypical pneumonia is caused by *Mycoplasma pneumoniae*. This bacterium is not part of the respiratory tract's normal microbiota and can cause epidemic disease outbreaks. Also known as walking pneumonia, ***mycoplasma pneumonia*** infections are common in crowded environments like college campuses and military bases. It is spread by aerosols formed when coughing or sneezing. The disease is often mild, with a low fever and persistent cough. These bacteria, which do not have cell walls, use a specialized attachment organelle to bind to ciliated cells. In the process, epithelial cells are damaged and the proper function of the cilia is hindered ([link](#)).

Mycoplasma grow very slowly when cultured. Therefore, penicillin and thallium acetate are added to agar to prevent the overgrowth by faster-

growing potential contaminants. Since *M. pneumoniae* does not have a cell wall, it is resistant to these substances. Without a cell wall, the microbial cells appear pleomorphic. *M. pneumoniae* infections tend to be self-limiting but may also respond well to macrolide antibiotic therapy. β -lactams, which target cell wall synthesis, are not indicated for treatment of infections with this pathogen.



The micrograph shows *Mycoplasma pneumoniae* using their specialized receptors to attach to epithelial cells in the trachea of an infected hamster. (credit: modification of work by American Society for Microbiology)

Chlamydial Pneumonias and Psittacosis

Chlamydial pneumonia can be caused by three different species of bacteria: *Chlamydophila pneumoniae* (formerly known as *Chlamydia pneumoniae*), *Chlamydophila psittaci* (formerly known as *Chlamydia psittaci*), and *Chlamydia trachomatis*. All three are obligate intracellular pathogens and cause mild to severe pneumonia and bronchitis. Of the three, *Chlamydophila pneumoniae* is the most common and is transmitted via respiratory droplets or aerosols. *C. psittaci* causes **psittacosis**, a zoonotic disease that primarily affects domesticated birds such as parakeets, turkeys, and ducks, but can be transmitted from birds to humans. Psittacosis is a relatively rare infection and is typically found in people who work with birds. *Chlamydia trachomatis*, the causative agent of the sexually transmitted disease chlamydia, can cause pneumonia in infants when the infection is passed from mother to baby during birth.

Diagnosis of chlamydia by culturing tends to be difficult and slow. Because they are intracellular pathogens, they require multiple passages through tissue culture. Recently, a variety of PCR- and serologically based tests have been developed to enable easier identification of these pathogens. Tetracycline and macrolide antibiotics are typically prescribed for treatment.

Health Care-Associated Pneumonia

A variety of opportunistic bacteria that do not typically cause respiratory disease in healthy individuals are common causes of health care-associated

pneumonia. These include *Klebsiella pneumoniae*, *Staphylococcus aureus*, and proteobacteria such as species of *Escherichia*, *Proteus*, and *Serratia*. Patients at risk include the elderly, those who have other preexisting lung conditions, and those who are immunocompromised. In addition, patients receiving supportive therapies such as intubation, antibiotics, and immunomodulatory drugs may also be at risk because these interventions disrupt the mucociliary escalator and other pulmonary defenses. Invasive medical devices such as catheters, medical implants, and ventilators can also introduce opportunistic pneumonia-causing pathogens into the body.

[\[footnote\]](#)

SM Koenig et al. “Ventilator-Associated Pneumonia: Diagnosis, Treatment, and Prevention.” *Clinical Microbiology Reviews* 19 no. 4 (2006):637–657.

Pneumonia caused by *K. pneumoniae* is characterized by lung necrosis and “currant jelly sputum,” so named because it consists of clumps of blood, mucus, and debris from the thick polysaccharide capsule produced by the bacterium. *K. pneumoniae* is often multidrug resistant. Aminoglycoside and cephalosporin are often prescribed but are not always effective. *Klebsiella pneumoniae* is frequently fatal even when treated.

Pseudomonas Pneumonia

Pseudomonas aeruginosa is another opportunistic pathogen that can cause serious cases of bacterial pneumonia in patients with cystic fibrosis (CF) and hospitalized patients assisted with artificial ventilators. This bacterium is extremely antibiotic resistant and can produce a variety of exotoxins. Ventilator-associated pneumonia with *P. aeruginosa* is caused by contaminated equipment that causes the pathogen to be aspirated into the lungs. In patients with CF, a genetic defect in the cystic fibrosis transmembrane receptor (CFTR) leads to the accumulation of excess dried mucus in the lungs. This decreases the effectiveness of the defensins and inhibits the mucociliary escalator. *P. aeruginosa* is known to infect more than half of all patients with CF. It adapts to the conditions in the patient’s lungs and begins to produce alginate, a viscous exopolysaccharide that inhibits the mucociliary escalator. Lung damage from the chronic

inflammatory response that ensues is the leading cause of mortality in patients with CF.[\[footnote\]](#)

R. Sordé et al. “Management of Refractory *Pseudomonas aeruginosa* Infection in Cystic Fibrosis.” *Infection and Drug Resistance* 4 (2011):31–41.

Note:

- What three pathogens are responsible for the most prevalent types of bacterial pneumonia?
- Which cause of pneumonia is most likely to affect young people?
- In what contexts does *Pseudomonas aeruginosa* cause pneumonia?

Note:

Part 2

John’s chest radiograph revealed an extensive consolidation in the right lung, and his sputum cultures revealed the presence of a gram-negative rod. His physician prescribed a course of the antibiotic clarithromycin. He also ordered the rapid influenza diagnostic tests (RIDTs) for type A and B influenza to rule out a possible underlying viral infection. Despite antibiotic therapy, John’s condition continued to deteriorate, so he was admitted to the hospital.

- What are some possible causes of pneumonia that would not have responded to the prescribed antibiotic?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Tuberculosis

Tuberculosis (TB) is one of the deadliest infectious diseases in human history. Although **tuberculosis** infection rates in the United States are extremely low, the CDC estimates that about one-third of the world's population is infected with *Mycobacterium tuberculosis*, the causal organism of TB, with 9.6 million new TB cases and 1.5 million deaths worldwide in 2014.[\[footnote\]](#)

Centers for Disease Control and Prevention. "Tuberculosis (TB). Data and Statistics." <http://www.cdc.gov/tb/statistics/default.htm>

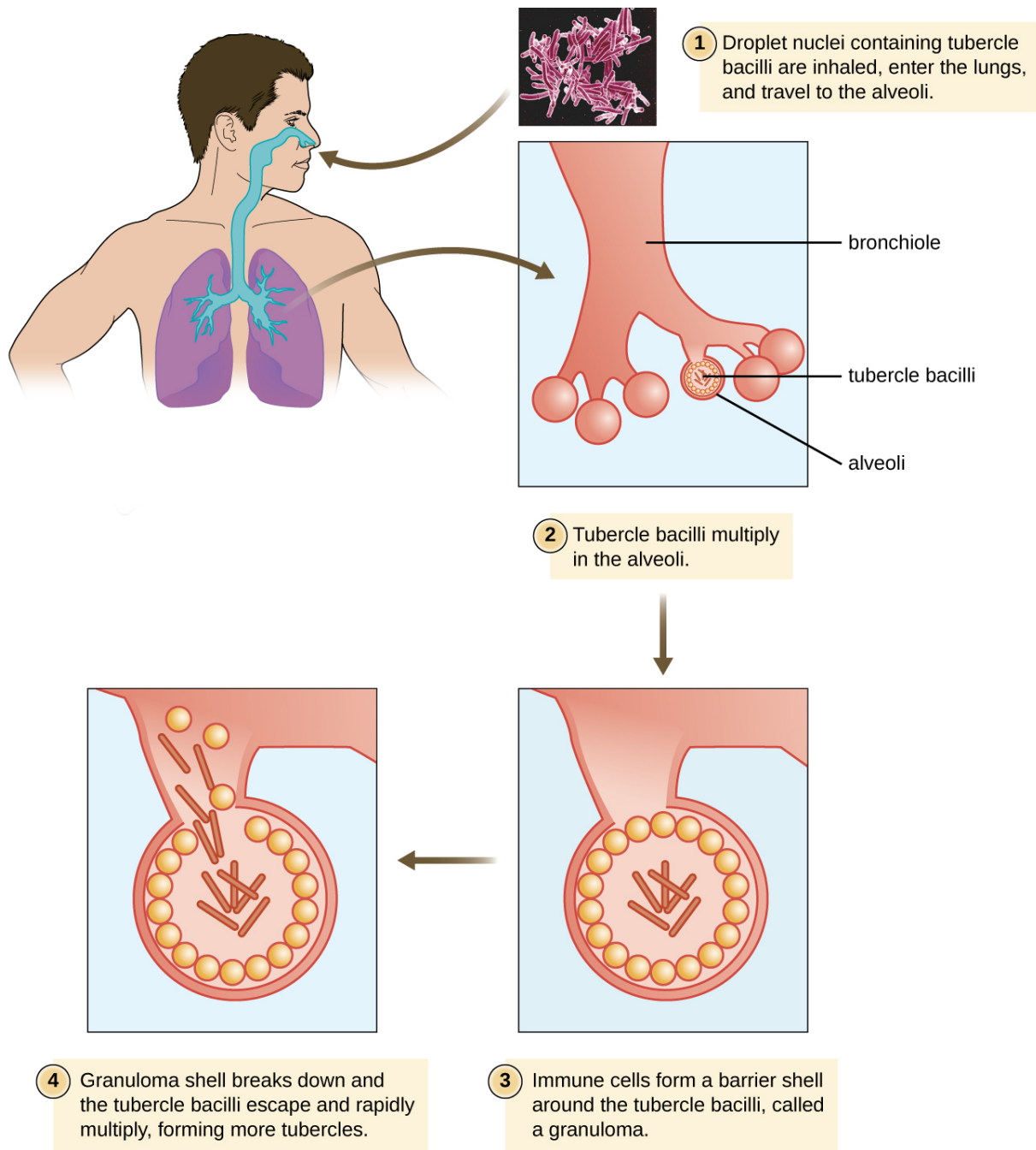
M. tuberculosis is an acid-fast, high G + C, gram-positive, nonspore-forming rod. Its cell wall is rich in waxy mycolic acids, which make the cells impervious to polar molecules. It also causes these organisms to grow slowly. *M. tuberculosis* causes a chronic granulomatous disease that can infect any area of the body, although it is typically associated with the lungs. *M. tuberculosis* is spread by inhalation of respiratory droplets or aerosols from an infected person. The infectious dose of *M. tuberculosis* is only 10 cells.[\[footnote\]](#)

D. Saini et al. "Ultra-Low Dose of *Mycobacterium tuberculosis* Aerosol Creates Partial Infection in Mice." *Tuberculosis* 92 no. 2 (2012):160–165.

After inhalation, the bacteria enter the alveoli ([\[link\]](#)). The cells are phagocytized by macrophages but can survive and multiply within these phagocytes because of the protection by the waxy mycolic acid in their cell walls. If not eliminated by macrophages, the infection can progress, causing an inflammatory response and an accumulation of neutrophils and macrophages in the area. Several weeks or months may pass before an immunological response is mounted by T cells and B cells. Eventually, the lesions in the alveoli become walled off, forming small round lesions called **tubercles**. Bacteria continue to be released into the center of the tubercles and the chronic immune response results in tissue damage and induction of apoptosis (programmed host-cell death) in a process called liquefaction. This creates a caseous center, or air pocket, where the aerobic *M. tuberculosis* can grow and multiply. Tubercles may eventually rupture and bacterial cells can invade pulmonary capillaries; from there, bacteria can spread through the bloodstream to other organs, a condition known as **miliary tuberculosis**. The rupture of tubercles also facilitates transmission of the bacteria to other individuals via droplet aerosols that exit the body in

coughs. Because these droplets can be very small and stay aloft for a long time, special precautions are necessary when caring for patients with TB, such as the use of face masks and negative-pressure ventilation and filtering systems.

Eventually, most lesions heal to form calcified **Ghon complexes**. These structures are visible on chest radiographs and are a useful diagnostic feature. But even after the disease has apparently ended, viable bacteria remain sequestered in these locations. Release of these organisms at a later time can produce **reactivation tuberculosis** (or secondary TB). This is mainly observed in people with alcoholism, the elderly, or in otherwise immunocompromised individuals ([\[link\]](#)).



In the infectious cycle of tuberculosis, the immune response of most infected individuals (approximately 90%) results in the formation of tubercles in which the infection is walled off.[\[footnote\]](#) The remainder will suffer progressive primary tuberculosis. The sequestered bacteria may be reactivated to form secondary tuberculosis in immunocompromised patients at a later time. (credit: modification of work by Centers for Disease Control and Prevention)

G. Kaplan et al. “*Mycobacterium tuberculosis* Growth at the Cavity Surface: A Microenvironment with Failed Immunity.” *Infection and Immunity* 71 no.12 (2003):7099–7108.

Because TB is a chronic disease, chemotherapeutic treatments often continue for months or years. Multidrug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains of *M. tuberculosis* are a growing clinical concern. These strains can arise due to misuse or mismanagement of antibiotic therapies. Therefore, it is imperative that proper multidrug protocols are used to treat these infections. Common antibiotics included in these mixtures are isoniazid, rifampin, ethambutol, and pyrazinamide.

A TB vaccine is available that is based on the so-called bacillus Calmette-Guérin (BCG) strain of *M. bovis* commonly found in cattle. In the United States, the BCG vaccine is only given to health-care workers and members of the military who are at risk of exposure to active cases of TB. It is used more broadly worldwide. Many individuals born in other countries have been vaccinated with BCG strain. BCG is used in many countries with a high prevalence of TB, to prevent childhood tuberculous meningitis and miliary disease.

The Mantoux tuberculin skin test ([\[link\]](#)) is regularly used in the United States to screen for potential TB exposure (see [Hypersensitivities](#)). However, prior vaccinations with the BCG vaccine can cause false-positive results. Chest radiographs to detect Ghon complex formation are required, therefore, to confirm exposure.



(a)



(b)

(a) The Mantoux skin test for tuberculosis involves injecting the subject with tuberculin protein derivative. The injection should initially produce a raised wheal. (b) The test should be read in 48–72 hours. A positive result is indicated by redness, swelling, or hardness; the size of the responding region is measured to determine the final result. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Note:

These short [animations](#) discuss the infection strategies of *Mycobacterium tuberculosis* and *Legionella pneumophila*.

Note:

- What characteristic of *Mycobacterium tuberculosis* allows it to evade the immune response?
- What happens to cause miliary tuberculosis?
- Explain the limitations of the Mantoux tuberculin skin test.

Pertussis (Whooping Cough)

The causative agent of **pertussis**, commonly called **whooping cough**, is *Bordetella pertussis*, a gram-negative coccobacillus. The disease is characterized by mucus accumulation in the lungs that leads to a long period of severe coughing. Sometimes, following a bout of coughing, a sound resembling a “whoop” is produced as air is inhaled through the inflamed and restricted airway—hence the name whooping cough. Although adults can be infected, the symptoms of this disease are most pronounced in infants and children. Pertussis is highly communicable through droplet transmission, so the uncontrollable coughing produced is an efficient means of transmitting the disease in a susceptible population.

Following inhalation, *B. pertussis* specifically attaches to epithelial cells using an adhesin, filamentous hemagglutinin. The bacteria then grow at the site of infection and cause disease symptoms through the production of exotoxins. One of the main virulence factors of this organism is an A-B exotoxin called the **pertussis toxin (PT)**. When PT enters the host cells, it increases the cyclic adenosine monophosphate (cAMP) levels and disrupts cellular signaling. PT is known to enhance inflammatory responses involving histamine and serotonin. In addition to PT, *B. pertussis* produces a tracheal cytotoxin that damages ciliated epithelial cells and results in accumulation of mucus in the lungs. The mucus can support the colonization and growth of other microbes and, as a consequence, secondary infections are common. Together, the effects of these factors produce the cough that characterizes this infection.

A pertussis infection can be divided into three distinct stages. The initial infection, termed the **catarrhal stage**, is relatively mild and unremarkable. The signs and symptoms may include nasal congestion, a runny nose, sneezing, and a low-grade fever. This, however, is the stage in which *B. pertussis* is most infectious. In the **paroxysmal stage**, mucus accumulation leads to uncontrollable coughing spasms that can last for several minutes and frequently induce vomiting. The paroxysmal stage can last for several weeks. A long **convalescence stage** follows the paroxysmal stage, during which time patients experience a chronic cough that can last for up to several months. In fact, the disease is sometimes called the 100-day cough.

In infants, coughing can be forceful enough to cause fractures to the ribs, and prolonged infections can lead to death. The CDC reported 20 pertussis-related deaths in 2012,[\[footnote\]](#) but that number had declined to five by 2015.[\[footnote\]](#)

Centers for Disease Control and Prevention. “2012 Final Pertussis Surveillance Report.” 2015.

<http://www.cdc.gov/pertussis/downloads/pertuss-surv-report-2012.pdf>. Accessed July 6, 2016.

Centers for Disease Control and Prevention. “2015 Provisional Pertussis Surveillance Report.” 2016.

<http://www.cdc.gov/pertussis/downloads/pertuss-surv-report-2015-provisional.pdf>. Accessed July 6, 2016.

During the first 2 weeks of infection, laboratory diagnosis is best performed by culturing the organism directly from a nasopharyngeal (NP) specimen collected from the posterior nasopharynx. The NP specimen is streaked onto Bordet-Gengou medium. The specimens must be transported to the laboratory as quickly as possible, even if transport media are used. Transport times of longer than 24 hours reduce the viability of *B. pertussis* significantly.

Within the first month of infection, *B. pertussis* can be diagnosed using PCR techniques. During the later stages of infection, pertussis-specific antibodies can be immunologically detected using an enzyme-linked immunosorbent assay (ELISA).

Pertussis is generally a self-limiting disease. Antibiotic therapy with erythromycin or tetracycline is only effective at the very earliest stages of disease. Antibiotics given later in the infection, and prophylactically to uninfected individuals, reduce the rate of transmission. Active vaccination is a better approach to control this disease. The DPT vaccine was once in common use in the United States. In that vaccine, the P component consisted of killed whole-cell *B. pertussis* preparations. Because of some adverse effects, that preparation has now been superseded by the DTaP and Tdap vaccines. In both of these new vaccines, the “aP” component is a pertussis toxoid.

Widespread vaccination has greatly reduced the number of reported cases and prevented large epidemics of pertussis. Recently, however, pertussis has begun to reemerge as a childhood disease in some states because of declining vaccination rates and an increasing population of susceptible children.

Note:

This [web page](#) contains an audio clip of the distinctive “whooping” sound associated with pertussis in infants.

This interactive [map](#) shows outbreaks of vaccine preventable diseases, including pertussis, around the world.

Note:

- What accounts for the mucus production in a pertussis infection?
- What are the signs and symptoms associated with the three stages of pertussis?
- Why is pertussis becoming more common in the United States?

Legionnaires Disease

An atypical pneumonia called **Legionnaires disease** (also known as legionellosis) is caused by an aerobic gram-negative bacillus, *Legionella pneumophila*. This bacterium infects free-living amoebae that inhabit moist environments, and infections typically occur from human-made reservoirs such as air-conditioning cooling towers, humidifiers, misting systems, and fountains. Aerosols from these reservoirs can lead to infections of susceptible individuals, especially those suffering from chronic heart or lung disease or other conditions that weaken the immune system.

When *L. pneumophila* bacteria enter the alveoli, they are phagocytized by resident macrophages. However, *L. pneumophila* uses a secretion system to insert proteins in the endosomal membrane of the macrophage; these proteins prevent lysosomal fusion, allowing *L. pneumophila* to continue to proliferate within the phagosome. The resulting respiratory disease can range from mild to severe pneumonia, depending on the status of the host's immune defenses. Although this disease primarily affects the lungs, it can also cause fever, nausea, vomiting, confusion, and other neurological effects.

Diagnosis of Legionnaires disease is somewhat complicated. *L. pneumophila* is a fastidious bacterium and is difficult to culture. In addition, since the bacterial cells are not efficiently stained with the Gram stain, other staining techniques, such as the Warthin-Starry silver-precipitate procedure, must be used to visualize this pathogen. A rapid diagnostic test has been developed that detects the presence of *Legionella* antigen in a patient's urine; results take less than 1 hour, and the test has high selectivity and specificity (greater than 90%). Unfortunately, the test only works for one serotype of *L. pneumophila* (type 1, the serotype responsible for most infections). Consequently, isolation and identification of *L. pneumophila* from sputum remains the defining test for diagnosis.

Once diagnosed, Legionnaire disease can be effectively treated with fluoroquinolone and macrolide antibiotics. However, the disease is sometimes fatal; about 10% of patients die of complications.[\[footnote\]](#) There is currently no vaccine available.

Centers for Disease Control and Prevention. "*Legionella* (Legionnaires' Disease and Pontiac Fever: Diagnosis, Treatment, and Complications)." <http://www.cdc.gov/legionella/about/diagnosis.html>. Accessed Sept 14, 2016.

Note:

- Why is Legionnaires disease associated with air-conditioning systems?
- How does *Legionella pneumophila* circumvent the immune system?

Q Fever

The zoonotic disease **Q fever** is caused by a rickettsia, *Coxiella burnetii*. The primary reservoirs for this bacterium are domesticated livestock such as cattle, sheep, and goats. The bacterium may be transmitted by ticks or through exposure to the urine, feces, milk, or amniotic fluid of an infected animal. In humans, the primary route of infection is through inhalation of contaminated farmyard aerosols. It is, therefore, largely an occupational disease of farmers. Humans are acutely sensitive to *C. burnetii*—the infective dose is estimated to be just a few cells.^[footnote] In addition, the organism is hardy and can survive in a dry environment for an extended time. Symptoms associated with acute Q fever include high fever, headache, coughing, pneumonia, and general malaise. In a small number of patients (less than 5%^[footnote]), the condition may become chronic, often leading to endocarditis, which may be fatal.

WD Tigertt et al. “Airborne Q Fever.” *Bacteriological Reviews* 25 no. 3 (1961):285–293.

Centers for Disease Control and Prevention. “Q fever. Symptoms, Diagnosis, and Treatment.” 2013.

<http://www.cdc.gov/qfever/symptoms/index.html>. Accessed July 6, 2016.

Diagnosing rickettsial infection by cultivation in the laboratory is both difficult and hazardous because of the easy aerosolization of the bacteria, so PCR and ELISA are commonly used. Doxycycline is the first-line drug to treat acute Q fever. In chronic Q fever, doxycycline is often paired with hydroxychloroquine.

Note:

Bacterial Diseases of the Respiratory Tract

Numerous pathogens can cause infections of the respiratory tract. Many of these infections produce similar signs and symptoms, but appropriate treatment depends on accurate diagnosis through laboratory testing. The

tables in [\[link\]](#) and [\[link\]](#) summarize the most important bacterial respiratory infections, with the latter focusing specifically on forms of bacterial pneumonia.

Bacterial Infections of the Respiratory Tract						
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Acute otitis media (AOM)	<i>Haemophilus influenzae</i> , <i>Streptococcus pneumoniae</i> , <i>Moraxella catarrhalis</i> , others	Earache, possible effusion; may cause fever, nausea, vomiting, diarrhea	Often a secondary infection; bacteria from respiratory tract become trapped in eustachian tube, cause infection	None	Cephalosporins, fluoroquinolones	None
Diphtheria	<i>Corynebacterium diphtheriae</i>	Pseudomembrane on throat, possibly leading to suffocation and death	Inhalation of respiratory droplets or aerosols from infected person	Identification of bacteria in throat swabs; PCR to detect diphtheria toxin in vitro	Erythromycin, penicillin, antitoxin produced in horses	DtaP, Tdap, DT, Td, DTP
Legionnaires disease	<i>Legionella pneumophila</i>	Cough, fever, muscle aches, headaches, nausea, vomiting, confusion; sometimes fatal	Inhalation of aerosols from contaminated water reservoirs	Isolation, using Warthin-Starry procedure, of bacteria in sputum	Fluoroquinolones, macrolides	None
Pertussis (whooping cough)	<i>Bordetella pertussis</i>	Severe coughing with "whoop" sound; chronic cough lasting several months; can be fatal in infants	Inhalation of respiratory droplets from infected person	Direct culture of throat swab, PCR, ELISA	Macrolides	DTaP, Tdap
Q fever	<i>Coxiella burnetii</i>	High fever, coughing, pneumonia, malaise; in chronic cases, potentially fatal endocarditis	Inhalation of aerosols of urine, feces, milk, or amniotic fluid of infected cattle, sheep, goats	PCR, ELISA	Doxycycline, hydroxychloroquine	None
Streptococcal pharyngitis, scarlet fever	<i>Streptococcus pyogenes</i>	Fever, sore throat, inflammation of pharynx and tonsils, petechiae, swollen lymph nodes; skin rash (scarlet fever), strawberry tongue	Direct contact, inhalation of respiratory droplets or aerosols from infected person	Direct culture of throat swab, rapid enzyme immunoassay	β -lactams	None
Tuberculosis	<i>Mycobacterium tuberculosis</i>	Formation of tubercles in lungs; rupture of tubercles, leading to chronic, bloody cough; healed tubercles (Ghon complexes) visible in radiographs; can be fatal	Inhalation of respiratory droplets or aerosols from infected person	Mantoux tuberculin skin test with chest radiograph to identify Ghon complexes	Isoniazid, rifampin, ethambutol, pyrazinamide	BCG

Bacterial Causes of Pneumonia						
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Chlamydial pneumonia	<i>Chlamydophila pneumoniae</i> , <i>C. psittaci</i> , <i>Chlamydia trachomatis</i>	Bronchitis; mild to severe respiratory distress	Inhalation of respiratory droplets or aerosols from infected person (<i>C. pneumoniae</i>); exposure to infected bird (<i>C. psittaci</i>); exposure in the birth canal (<i>Chlamydia trachomatis</i>)	Tissue culture, PCR	Tetracycline, macrolides	None
<i>Haemophilus</i> pneumonia	<i>Haemophilus influenzae</i>	Cough, fever or low body temperature, chills, chest pain, headache, fatigue	Inhalation of respiratory droplets or aerosols from infected person or asymptomatic carrier	Culture on chocolate agar, serotyping of blood or cerebrospinal fluid samples	Cephalosporins, fluoroquinolones	Hib
<i>Klebsiella</i> pneumonia	<i>Klebsiella pneumoniae</i> , others	Lung necrosis, "currant jelly" sputum; often fatal	Health care associated; bacteria introduced via contaminated ventilators, intubation, or other medical equipment	Culture, PCR	Multidrug resistant; antibiotic susceptibility testing necessary	None
Mycoplasma pneumonia (walking pneumonia)	<i>Mycoplasma pneumoniae</i>	Low fever, persistent cough	Inhalation of respiratory droplets or aerosols from infected person	Culture with penicillin, thallium acetate	Macrolides	None
Pneumococcal pneumonia	<i>Streptococcus pneumoniae</i>	Productive cough, bloody sputum, fever, chills, chest pain, respiratory distress	Direct contact with respiratory secretions	Gram stain, blood agar culture with optichin and sodium deoxycholate, quellung reaction	β -lactams, macrolides, fluoroquinolones	Pneumococcal conjugate vaccine (PCV13), pneumococcal polysaccharide vaccine (PPSV23)
<i>Pseudomonas</i> pneumonia	<i>Pseudomonas aeruginosa</i>	Viscous fluid and chronic inflammation of lungs; often fatal	Health care associated; bacteria introduced via contaminated ventilators; also frequently affects patients with cystic fibrosis	Culture from sputum or other body fluid	Multidrug resistant; antibiotic susceptibility testing necessary	None

Key Concepts and Summary

- A wide variety of bacteria can cause respiratory diseases; most are treatable with antibiotics or preventable with vaccines.
- *Streptococcus pyogenes* causes **strep throat**, an infection of the pharynx that also causes high fever and can lead to **scarlet fever**, **acute rheumatic fever**, and **acute glomerulonephritis**.
- **Acute otitis media** is an infection of the middle ear that may be caused by several bacteria, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. The infection can block the eustachian tubes, leading to **otitis media with effusion**.
- **Diphtheria**, caused by *Corynebacterium diphtheriae*, is now a rare disease because of widespread vaccination. The bacteria produce exotoxins that kill cells in the pharynx, leading to the formation of a **pseudomembrane**; and damage other parts of the body.
- **Bacterial pneumonia** results from infections that cause inflammation and fluid accumulation in the alveoli. It is most commonly caused by *S. pneumoniae* or *H. influenzae*. The former is commonly multidrug resistant.
- **Mycoplasma pneumonia** results from infection by *Mycoplasma pneumoniae*; it can spread quickly, but the disease is mild and self-limiting.
- **Chlamydial pneumonia** can be caused by three pathogens that are obligate intracellular parasites. *Chlamydophila pneumoniae* is typically transmitted from an infected person, whereas *C. psittaci* is typically transmitted from an infected bird. *Chlamydia trachomatis*, may cause pneumonia in infants.
- Several other bacteria can cause pneumonia in immunocompromised individuals and those with cystic fibrosis.
- **Tuberculosis** is caused by *Mycobacterium tuberculosis*. Infection leads to the production of protective **tubercles** in the alveoli and calcified **Ghon complexes** that can harbor the bacteria for a long time. Antibiotic-resistant forms are common and treatment is typically long term.
- **Pertussis** is caused by *Bordetella pertussis*. Mucus accumulation in the lungs leads to prolonged severe coughing episodes (whooping

cough) that facilitate transmission. Despite an available vaccine, outbreaks are still common.

- **Legionnaires disease** is caused by infection from environmental reservoirs of the *Legionella pneumophila* bacterium. The bacterium is endocytic within macrophages and infection can lead to pneumonia, particularly among immunocompromised individuals.
- **Q fever** is caused by *Coxiella burnetii*, whose primary hosts are domesticated mammals (zoonotic disease). It causes pneumonia primarily in farm workers and can lead to serious complications, such as endocarditis.

Multiple Choice

Exercise:

Problem:

Which of the following does not involve a bacterial exotoxin?

- A. diphtheria
- B. whooping cough
- C. scarlet fever
- D. Q fever

Solution:

D

Exercise:

Problem: What disease is caused by *Coxiella burnetii*?

- A. Q fever
 - B. tuberculosis
 - C. diphtheria
 - D. walking pneumonia
-

Solution:

A

Exercise:

Problem:

In which stage of pertussis is the characteristic whooping sound made?

- A. convalescence
- B. catarrhal
- C. paroxysmal
- D. prodromal

Solution:

C

Exercise:

Problem: What is the causative agent of Q fever?

- A. *Coxiella burnetii*
- B. *Chlamydophila psittaci*
- C. *Mycoplasma pneumoniae*
- D. *Streptococcus pyogenes*

Solution:

A

Exercise:

Problem: Which of these microbes causes “walking pneumonia”?

- A. *Klebsiella pneumoniae*
- B. *Streptococcus pneumoniae*

- C. *Mycoplasma pneumoniae*
D. *Chlamydophila pneumoniae*
-

Solution:

C

Fill in the Blank

Exercise:

Problem:

Calcified lesions called _____ form in the lungs of patients with TB.

Solution:

Ghon complexes

Exercise:

Problem: An inflammation of the middle ear is called _____.

Solution:

otitis media

Exercise:

Problem:

The _____ is used to serologically identify *Streptococcus pneumoniae* isolates.

Solution:

quellung reaction

Exercise:

Problem:

_____ is a zoonotic infection that can be contracted by people who handle birds.

Solution:

Psittacosis

Exercise:**Problem:**

The main virulence factor involved in scarlet fever is the _____.

Solution:

erythrogenic toxin

Short Answer**Exercise:****Problem:**

Name three bacteria that commonly cause pneumonia. Which is the most common cause?

Exercise:**Problem:**

How does smoking make an individual more susceptible to infections?

Exercise:**Problem:**

How does the diphtheria pathogen form a pseudomembrane?

Critical Thinking

Exercise:

Problem:

Why might β -lactam antibiotics be ineffective against *Mycoplasma pneumoniae* infections?

Exercise:

Problem:

Why is proper antibiotic therapy especially important for patients with tuberculosis?

Viral Infections of the Respiratory Tract

LEARNING OBJECTIVES

- Identify the most common viruses that can cause infections of the upper and lower respiratory tract
- Compare the major characteristics of specific viral diseases of the respiratory tract

Viruses are the most frequent cause of respiratory tract infections. Unlike the bacterial pathogens, we have few effective therapies to combat viral respiratory infections. Fortunately, many of these diseases are mild and self-limiting. A few respiratory infections manifest their primary symptoms at other locations in the body.

The Common Cold

The **common cold** is a generic term for a variety of mild viral infections of the nasal cavity. More than 200 different viruses are known to cause the common cold. The most common groups of cold viruses include rhinoviruses, coronaviruses, and adenoviruses. These infections are widely disseminated in the human population and are transmitted through direct contact and droplet transmission. Coughing and sneezing efficiently produce infectious aerosols, and rhinoviruses are known to persist on environmental surfaces for up to a week.[\[footnote\]](#)

AG L'Huillier et al. "Survival of Rhinoviruses on Human Fingers." *Clinical Microbiology and Infection* 21, no. 4 (2015):381–385.

Viral contact with the nasal mucosa or eyes can lead to infection. Rhinoviruses tend to replicate best between 33 °C (91.4 °F) and 35 °C (95 °F), somewhat below normal body temperature (37 °C [98.6 °F]). As a consequence, they tend to infect the cooler tissues of the nasal cavities. Colds are marked by an irritation of the mucosa that leads to an inflammatory response. This produces common signs and symptoms such as nasal excess nasal secretions (runny nose), congestion, sore throat, coughing, and sneezing. The absence of high fever is typically used to differentiate common colds from other viral infections, like influenza. Some colds may progress to cause otitis media, pharyngitis, or laryngitis, and patients may also experience headaches and body aches. The disease, however, is self-limiting and typically resolves within 1–2 weeks.

There are no effective antiviral treatments for the common cold and antibacterial drugs should not be prescribed unless secondary bacterial infections have been established. Many of the viruses that cause colds are related, so immunity develops throughout life. Given the number of viruses that cause colds, however, individuals are never likely to develop immunity to all causes of the common cold.

Note:

- How are colds transmitted?
- What is responsible for the symptoms of a cold?

Note:

Part 3

Since antibiotic treatment had proven ineffective, John's doctor suspects that a viral or fungal pathogen may be the culprit behind John's case of pneumonia. Another possibility is that John could have an antibiotic-resistant bacterial infection that will require a different antibiotic or combination of antibiotics to clear.

The RIDT tests both came back negative for type A and type B influenza. However, the diagnostic laboratory identified the sputum isolate as *Legionella pneumophila*. The doctor ordered tests of John's urine and, on the second day after his admission, results of an enzyme immunoassay (EIA) were positive for the *Legionella* antigen. John's doctor added levofloxacin to his antibiotic therapy and continued to monitor him. The doctor also began to ask John where he had been over the past 10 to 14 days.

- Do negative RIDT results absolutely rule out influenza virus as the etiologic agent? Why or why not?
- What is John's prognosis?

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Influenza

Commonly known as the flu, **influenza** is a common viral disease of the lower respiratory system caused by an orthomyxovirus. Influenza is pervasive worldwide and causes 3,000–50,000 deaths each year in the United States. The annual mortality rate can vary greatly depending on the virulence of the strain(s) responsible for seasonal epidemics. [\[footnote\]](#) Centers for Disease Control and Prevention. “Estimating Seasonal Influenza-Associated Deaths in the United States: CDC Study Confirms Variability of Flu.” 2016. http://www.cdc.gov/flu/about/disease/us_flu-related_deaths.htm. Accessed July 6, 2016.

Influenza infections are most typically characterized by fever, chills, and body aches. This is followed by symptoms similar to the common cold that may last a week or more. [\[link\]](#) compares the signs and symptoms of influenza and the common cold.

Comparing the Common Cold and Influenza		
Sign/Symptom	Common Cold	Influenza
Fever	Low (37.2 °C [99 °F])	High (39 °C [102.2 °F])
Headache	Common	Common
Aches and pains	Mild	Severe
Fatigue	Slight	Severe
Nasal congestion	Common	Rare
Sneezing	Common	Rare

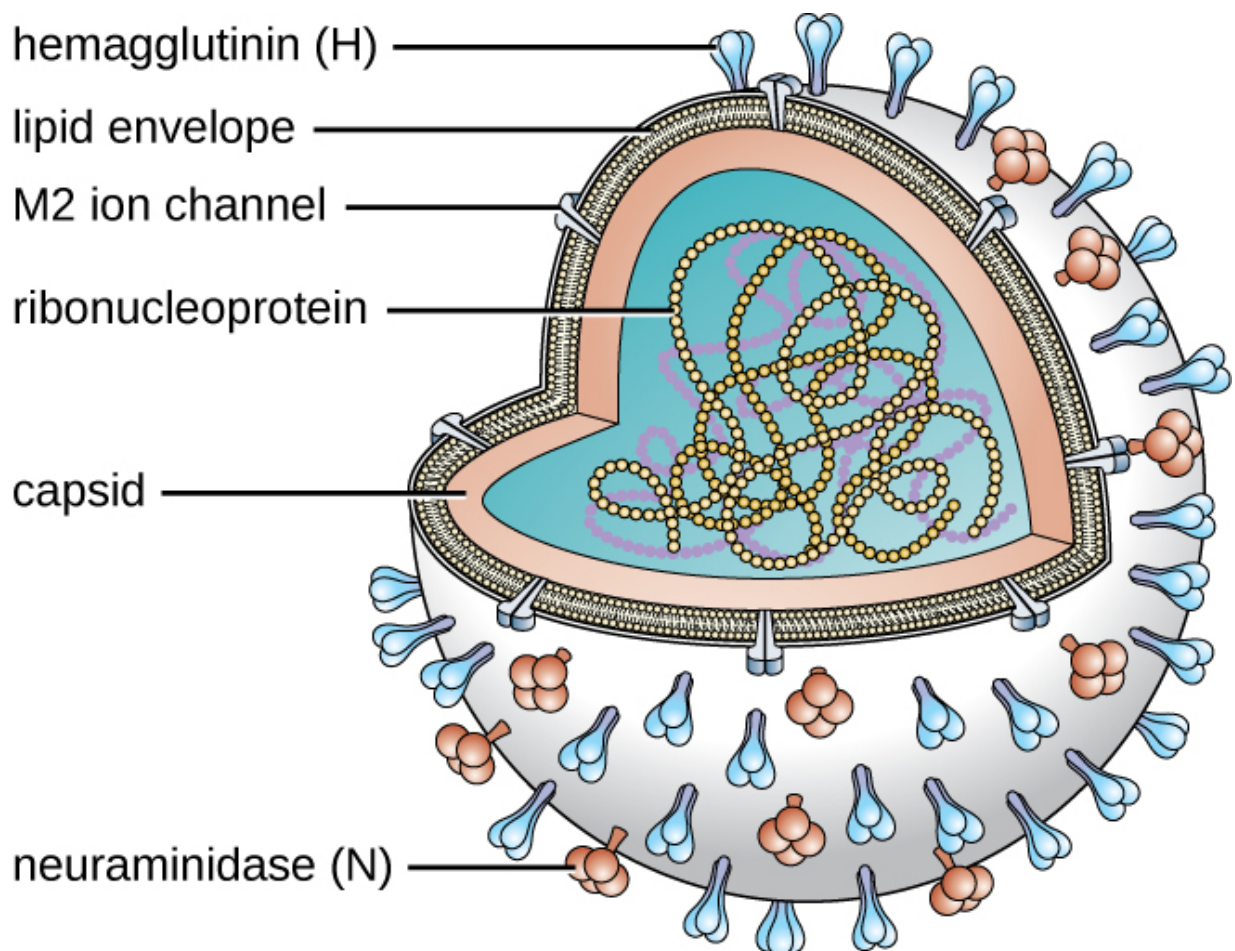
In general, influenza is self-limiting. However, serious cases can lead to pneumonia and other complications that can be fatal. Such cases are more common in the very young and the elderly; however, certain strains of influenza virus (like the 1918–1919 variant discussed later in this chapter) are more lethal to young adults than to the very young or old. Strains that affect young adults are believed to involve a cytokine storm—a positive feedback loop that forms between cytokine production and leukocytes. This cytokine storm produces an acute inflammatory response that leads to rapid fluid accumulation in the lungs, culminating in pulmonary failure. In such cases, the ability to mount a vigorous immune response is actually detrimental to the patient. The very young and very old are less susceptible to this effect because their immune systems are less robust.

A complication of influenza that occurs primarily in children and teenagers is **Reye syndrome**. This sequela causes swelling in the liver and brain, and may progress to neurological damage, coma, or death. Reye syndrome may follow other viral infections, like chickenpox, and has been associated with

the use of aspirin. For this reason, the CDC and other agencies recommend that aspirin and products containing aspirin never be used to treat viral illnesses in children younger than age 19 years.[\[footnote\]](#)

ED Belay et al. “Reye’s Syndrome in the United States From 1981 Through 1997.” *New England Journal of Medicine* 340 no. 18 (1999):1377–1382.

The influenza virus is primarily transmitted by direct contact and inhalation of aerosols. The RNA genome of this virus exists as seven or eight segments, each coated with ribonucleoprotein and encoding one or two specific viral proteins. The influenza virus is surrounded by a lipid membrane envelope, and two of the main antigens of the influenza virus are the spike proteins hemagglutinin (H) and neuraminidase (N), as shown in [\[link\]](#). These spike proteins play important roles in the viral infectious cycle.



The illustration shows the structure of an influenza virus. The viral envelope is studded with copies of the proteins neuraminidase and hemagglutinin, and surrounds the individual seven or eight RNA genome segments. (credit: modification of work by Dan Higgins, Centers for Disease Control and Prevention)

Following inhalation, the influenza virus uses the hemagglutinin protein to bind to sialic acid receptors on host respiratory epithelial cells. This facilitates endocytosis of the viral particle. Once inside the host cell, the negative strand viral RNA is replicated by the viral RNA polymerase to form mRNA, which is translated by the host to produce viral proteins. Additional viral RNA molecules are transcribed to produce viral genomic RNA, which assemble with viral proteins to form mature virions. Release of the virions from the host cell is facilitated by viral neuraminidase, which cleaves sialic-acid receptors to allow progeny viruses to make a clean exit when budding from an infected cell.

There are three genetically related influenza viruses, called A, B, and C. The influenza A viruses have different subtypes based on the structure of their hemagglutinin and neuraminidase proteins. There are currently 18 known subtypes of hemagglutinin and 11 known subtypes of neuraminidase. Influenza viruses are serologically characterized by the type of H and N proteins that they possess. Of the nearly 200 different combinations of H and N, only a few, such as the H1N1 strain, are associated with human disease. The influenza viruses A, B, and C make up three of the five major groups of orthomyxoviruses. The differences between the three types of influenza are summarized in [\[link\]](#). The most virulent group is the influenza A viruses, which cause seasonal pandemics of influenza each year. Influenza A virus can infect a variety of animals, including pigs, horses, pigs, and even whales and dolphins. Influenza B virus is less virulent and is sometimes associated with epidemic outbreaks. Influenza C virus generally produces the mildest disease symptoms and is rarely connected with epidemics. Neither influenza B virus nor influenza C virus has significant animal reservoirs.

The Three Major Groups of Influenza Viruses			
	Influenza A virus	Influenza B virus	Influenza C virus
Severity	Severe	Moderate	Mild
Animal reservoir	Yes	No	No
Genome segments	8	8	7
Population spread	Epidemic and pandemic	Epidemic	Sporadic
Antigenic variation	Shift/drift	Drift	Drift

Influenza virus infections elicit a strong immune response, particularly to the hemagglutinin protein, which would protect the individual if they encountered the same virus. Unfortunately, the antigenic properties of the virus change relatively rapidly, so new strains are evolving that immune systems previously challenged by influenza virus cannot recognize. When an influenza virus gains a new hemagglutinin or neuraminidase type, it is able to evade the host's immune response and be successfully transmitted, often leading to an epidemic.

There are two mechanisms by which these evolutionary changes may occur. The mechanisms of antigen drift and antigenic shift for influenza virus have been described in [Virulence Factors of Bacterial and Viral Pathogens](#). Of these two genetic processes, it is viruses produced by antigenic shift that have the potential to be extremely virulent because individuals previously infected by other strains are unlikely to produce any protective immune response against these novel variants.

The most lethal influenza pandemic in recorded history occurred from 1918 through 1919. Near the end of World War I, an antigenic shift involving the recombination of avian and human viruses is thought to have produced a new H1N1 virus. This strain rapidly spread worldwide and is commonly claimed to have killed as many as 40 million to 50 million people—more than double the number killed in the war. Although referred to as the Spanish flu, this disease is thought to have originated in the United States. Regardless of its source, the conditions of World War I greatly contributed to the spread of this disease. Crowding, poor sanitation, and rapid mobilization of large numbers of personnel and animals facilitated the dissemination of the new virus once it appeared.

Several of the most important influenza pandemics of modern times have been associated with antigenic shifts. A few of these are summarized in [\[link\]](#).

Historical Influenza Outbreaks [footnote] [footnote] [footnote] CE Mills et al. “Transmissibility of 1918 Pandemic Influenza.” <i>Nature</i> 432, no. 7019 (2004):904–906. E. Tognotti. “Influenza Pandemics: A Historical Retrospect.” <i>Journal of Infection in Developing Countries</i> 3, no. 5 (2009):331–334. FS Dawood et al. “Estimated Global Mortality Associated with the First 12 Months of 2009 Pandemic Influenza A H1N1 Virus Circulation: A Modelling Study.” <i>The Lancet Infectious Diseases</i> 12, no. 9 (2012):687–695.			
Years	Common Name	Serotype	Estimated Number of Deaths
1918–1919	Spanish flu	H1N1	20,000,000–40,000,000

1957– 1958	Asian flu	N2N2	1,000,000–2,000,000
1968– 1969	Hong Kong flu	H3N2	1,000,000–3,000,000
2009– 2010	Swine flu	H1N1/09	152,000–575,000

Laboratory diagnosis of influenza is typically performed using a variety of RIDTs. These tests are inoculated by point-of-care personnel and give results within 15–20 minutes. Unfortunately, these tests have variable sensitivity and commonly yield false-negative results. Other tests include hemagglutination of erythrocytes (due to hemagglutinin action) or complement fixation. Patient serum antibodies against influenza viruses can also be detected in blood samples. Because influenza is self-limiting disease, diagnosis through these more time-consuming and expensive methods is not typically used.

Three drugs that inhibit influenza neuraminidase activity are available: inhaled zanamivir, oral oseltamivir, and intravenous peramivir. If taken at the onset of symptoms, these drugs can shorten the course of the disease. These drugs are thought to impair the ability of the virus to efficiently exit infected host cells. A more effective means of controlling influenza outbreaks, though, is vaccination. Every year, new influenza vaccines are developed to be effective against the strains expected to be predominant. This is determined in February by a review of the dominant strains around the world from a network of reporting sites; their reports are used to generate a recommendation for the vaccine combination for the following winter in the northern hemisphere. In September, a similar recommendation is made for the winter in the southern hemisphere.[\[footnote\]](#) These recommendations are used by vaccine manufacturers to formulate each year's vaccine. In most cases, three or four viruses are selected—the two most prevalent influenza A strains and one or two influenza B strains. The chosen strains are typically cultivated in eggs and used to produce either an inactivated or a live attenuated vaccine (e.g., FluMist). For individuals 18

years or older with an allergy to egg products, a recombinant egg-free trivalent vaccine is available. Most of the influenza vaccines over the past decade have had an effectiveness of about 50%.[\[footnote\]](#)

World Health Organization. “WHO Report on Global Surveillance of Epidemic-Prone Infectious Diseases.” 2000.

<http://www.who.int/csr/resources/publications/surveillance/Influenza.pdf>. Accessed July 6, 2016.

Centers of Disease Control and Prevention. “Vaccine Effectiveness - How Well Does the Flu Vaccine Work?” 2016.

<http://www.cdc.gov/flu/about/qa/vaccineeffect.htm>. Accessed July 6, 2016.

Note:

Flu Pandemic

During the spring of 2013, a new strain of H7N9 influenza was reported in China. A total of 132 people were infected. Of those infected, 44 (33%) died. A genetic analysis of the virus suggested that this strain arose from the reassortment of three different influenza viruses: a domestic duck H7N3 virus, a wild bird H7N9 virus, and a domestic poultry H9N2 virus. The virus was detected in the Chinese domestic bird flocks and contact with this reservoir is thought to have been the primary source of infection. This strain of influenza was not able to spread from person to person. Therefore, the disease did not become a global problem. This case does, though, illustrate the potential threat that influenza still represents. If a strain like the H7N9 virus were to undergo another antigenic shift, it could become more communicable in the human population. With a mortality rate of 33%, such a pandemic would be disastrous. For this reason, organizations like the World Health Organization and the Centers for Disease Control and Prevention keep all known influenza outbreaks under constant surveillance.

Note:

- Compare the severity of the three types of influenza viruses.
- Why must new influenza vaccines be developed each year?

Viral Pneumonia

Viruses cause fewer cases of pneumonia than bacteria; however, several viruses can lead to pneumonia in children and the elderly. The most common sources of viral pneumonia are adenoviruses, influenza viruses, parainfluenza viruses, and respiratory syncytial viruses. The signs and symptoms produced by these viruses can range from mild cold-like symptoms to severe cases of pneumonia, depending on the virulence of the virus strain and the strength of the host defenses of the infected individual. Occasionally, infections can result in otitis media.

Respiratory syncytial virus (RSV) infections are fairly common in infants; most people have been infected by the age of 2 years. During infection, a viral surface protein causes host cells to fuse and form multinucleated giant cells called **syncytia**. There are no specific antiviral therapies or vaccines available for viral pneumonia. In adults, these infections are self-limiting, resemble the common cold, and tend to resolve uneventfully within 1 or 2 weeks. Infections in infants, however, can be life-threatening. RSV is highly contagious and can be spread through respiratory droplets from coughing and sneezing. RSV can also survive for a long time on environmental surfaces and, thus, be transmitted indirectly via fomites.

Note:

- Who is most likely to contract viral pneumonia?
- What is the recommended treatment for viral pneumonia?

SARS and MERS

Severe acute respiratory syndrome (**SARS**) and Middle East respiratory syndrome (**MERS**) are two acute respiratory infections caused by coronaviruses. In both cases, these are thought to be zoonotic infections. Bats and civet cats are thought to have been the reservoirs for SARS; camels seem to be the reservoir for MERS.

SARS originated in southern China in the winter of 2002 and rapidly spread to 37 countries. Within about 1 year, more than 8,000 people experienced influenza-like symptoms and nearly 800 people died. The rapid spread and severity of these infections caused grave concern at the time. However, the outbreak was controlled in 2003 and no further cases of SARS have been recorded since 2004.[\[footnote\]](#) Signs and symptoms of SARS include high fever, headache, body aches, and cough, and most patients will develop pneumonia.

Y. Huang. “The SARS Epidemic and Its Aftermath in China: A Political Perspective.” In *Learning from SARS: Preparing for the Next Disease Outbreak*. Edited by S. Knobler et al. Washington, DC: National Academies Press; 2004. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK92479/>

MERS was first reported in Saudi Arabia in 2013. Although some infected individuals will be asymptomatic or have mild cold-like symptoms, most will develop a high fever, aches, cough and a severe respiratory infection that can progress to pneumonia. As of 2015, over 1,300 people in 27 countries have been infected. About 500 people have died. There are no specific treatments for either MERS or SARS. In addition, no vaccines are currently available. Several recombinant vaccines, however, are being developed.

Note:

- What is the cause of SARS?
- What are the signs and symptoms of MERS?

Viral Respiratory Diseases Causing Skin Rashes

Measles, rubella (German measles), and chickenpox are three important viral diseases often associated with skin rashes. However, their symptoms are systemic, and because their portal of entry is the respiratory tract, they can be considered respiratory infections.

Measles (Rubeola)

The measles virus (MeV) causes the highly contagious disease **measles**, also known as rubeola, which is a major cause of childhood mortality worldwide. Although vaccination efforts have greatly reduced the incidence of measles in much of the world, epidemics are still common in unvaccinated populations in certain countries. [\[footnote\]](#)

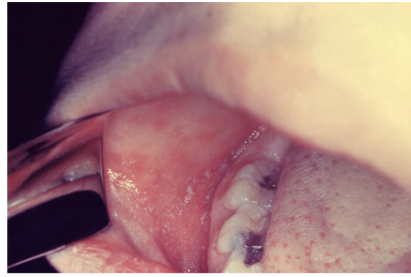
Centers for Disease Control and Prevention. “Global Health - Measles, Rubella, and CRS, Eliminating Measles, Rubella & Congenital Rubella Syndrome (CRS) Worldwide.” 2015.

<http://www.cdc.gov/globalhealth/measles/>. Accessed July 7, 2016.

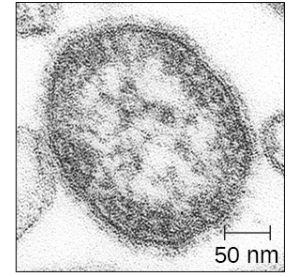
The measles virus is a single-stranded, negative-strand RNA virus and, like the influenza virus, it possesses an envelope with spikes of embedded hemagglutinin. The infection is spread by direct contact with infectious secretions or inhalation of airborne droplets spread by breathing, coughing, or sneezing. Measles is initially characterized by a high fever, conjunctivitis, and a sore throat. The virus then moves systemically through the bloodstream and causes a characteristic rash. The measles rash initially forms on the face and later spreads to the extremities. The red, raised macular rash will eventually become confluent and can last for several days. At the same time, extremely high fevers (higher than 40.6 °C [105 °F]) can occur. Another diagnostic sign of measles infections is **Koplik’s spots**, white spots that form on the inner lining of inflamed cheek tissues ([\[link\]](#)).



(a)



(b)



(c)

(a) Measles typically presents as a raised macular rash that begins on the face and spreads to the extremities. (b) Koplik's spots on the oral mucosa are also characteristic of measles. (c) A thin-section transmission electron micrograph of a measles virion. (credit a, b, c: modification of work by Centers for Disease Control and Prevention)

Although measles is usually self-limiting, it can lead to pneumonia, encephalitis, and death. In addition, the inhibition of immune system cells by the measles virus predisposes patients to secondary infections. In severe infections with highly virulent strains, measles fatality rates can be as high as 10% to 15%. There were more than 145,000 measles deaths (mostly young children) worldwide in 2013.[\[footnote\]](#)

World Health Organization. "Measles Factsheet." 2016.

<http://www.who.int/mediacentre/factsheets/fs286/en/>. Accessed July 7, 2016.

The preliminary diagnosis of measles is typically based on the appearance of the rash and Koplik's spots. Hemagglutination inhibition tests and serological tests may be used to confirm measles infections in low-prevalence settings.

There are no effective treatments for measles. Vaccination is widespread in developed countries as part of the measles, mumps, and rubella (MMR) vaccine. As a result, there are typically fewer than 200 cases of measles in the United States annually.[\[footnote\]](#) When it is seen, it is often associated with children who have not been vaccinated.

Centers for Disease Control and Prevention. "Measles Cases and Outbreaks." 2016. <http://www.cdc.gov/measles/cases-outbreaks.html>. Accessed July 7, 2016.

Note:

Preventable Measles Outbreaks

In December 2014, a measles epidemic began at Disneyland in southern California. Within just 4 months, this outbreak affected 134 people in 24 states.[\[footnote\]](#) Characterization of the virus suggests that an unidentified infected individual brought the disease to the United States from the Philippines, where a similar virus had sickened more than 58,000 people and killed 110.[\[footnote\]](#) Measles is highly communicable, and its spread at Disneyland may have been facilitated by the low vaccination rate in some communities in California.[\[footnote\]](#)

Ibid.

World Health Organization. "Measles-Rubella Bulletin." Manila, Philippines; Expanded Programme on Immunization Regional Office for the Western Pacific World Health Organization; 9 no. 1 (2015). <http://www.wpro.who.int/immunization/documents/mrbulletinvol9issue1.pdf>

M. Bloch et al. "Vaccination Rates for Every Kindergartener in California." *The New York Times* February 6, 2015.

http://www.nytimes.com/interactive/2015/02/06/us/california-measles-vaccines-map.html?_r=1. Accessed July 7, 2016.

Several factors could conceivably lead to a strong comeback of measles in the U.S. Measles is still an epidemic disease in many locations worldwide. Air travel enables infected individuals to rapidly translocate these infections globally. Compounding this problem, low vaccination rates in some local areas in the United States (such as in Amish communities) provide populations of susceptible hosts for the virus to establish itself. Finally, measles has been a low-prevalence infection in the U.S. for some time. As a consequence, physicians are not as likely to recognize the initial symptoms and make accurate diagnoses. Until vaccination rates become high enough to ensure herd immunity, measles is likely to be an ongoing problem in the United States.

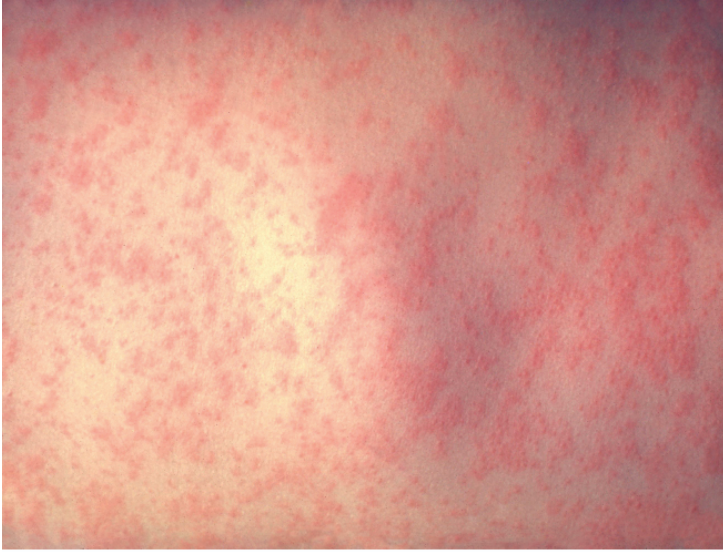
Rubella (German Measles)

Rubella, or the German measles, is a relatively mild viral disease that produces a rash somewhat like that caused by the measles, even though the two diseases are unrelated. The rubella virus is an enveloped RNA virus that can be found in the respiratory tract. It is transmitted from person to person in aerosols produced by coughing or sneezing. Nearly half of all infected people remain asymptomatic. However, the virus is shed and spread by asymptomatic carriers. Like rubeola, **rubella** begins with a facial rash that spreads to the extremities ([\[link\]](#)). However, the rash is less intense, shorter lived (2–3 days), not associated with Koplik’s spots, and the resulting fever is lower (101 °F [38.3 °C]).

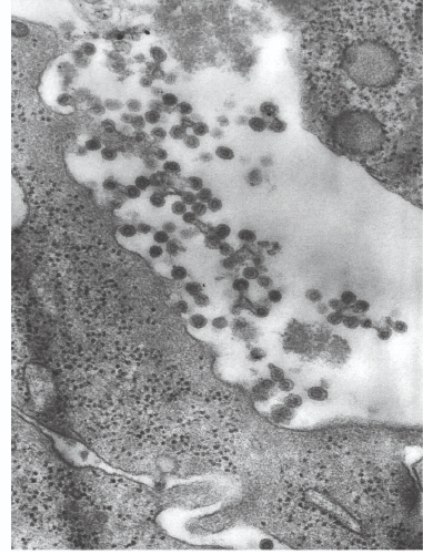
Congenital rubella syndrome is the most severe clinical complication of the German measles. This occurs if a woman is infected with rubella during pregnancy. The rubella virus is **teratogenic**, meaning it can cause developmental defects if it crosses the placenta during pregnancy. There is a very high incidence of stillbirth, spontaneous abortion, or congenital birth defects if the mother is infected before 11 weeks of pregnancy and 35% if she is infected between weeks 13–16; after this time the incidence is low. [\[footnote\]](#) For this reason, prenatal screening for rubella is commonly practiced in the United States. Postnatal infections are usually self-limiting and rarely cause severe complications.

E. Miller et al. “Consequences of Confirmed Maternal Rubella at Successive Stages of Pregnancy.” *The Lancet* 320, no. 8302 (1982):781–784.

Like measles, the preliminary diagnosis of rubella is based on the patient’s history, vaccination records, and the appearance of the rash. The diagnosis can be confirmed by hemagglutinin inhibition assays and a variety of other immunological techniques. There are no antiviral therapies for rubella, but an effective vaccine (MMR) is widely available. Vaccination efforts have essentially eliminated rubella in the United States; fewer than a dozen cases are reported in a typical year.



(a)



(b)

(a) This photograph shows the appearance of the German measles (rubella) rash. Note that this is less intense than the rash of measles and the lesions are not confluent. (b) This transmission electron micrograph shows rubella virus virions just budding from a host cell. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Chickenpox and Shingles

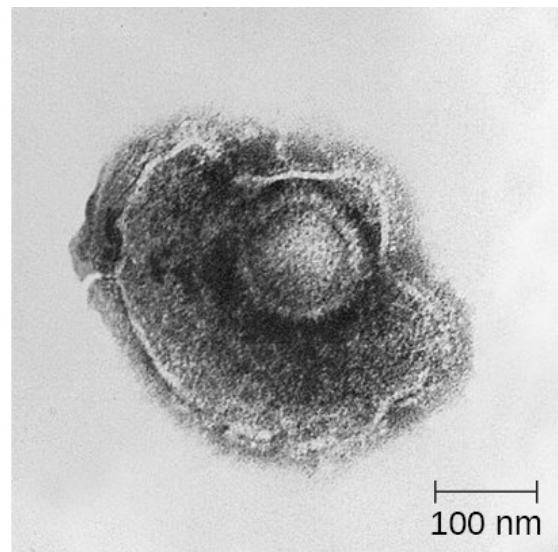
Chickenpox, also known as varicella, was once a common viral childhood disease. The causative agent of **chickenpox**, the varicella-zoster virus, is a member of the herpesvirus family. In children, the disease is mild and self-limiting, and is easily transmitted by direct contact or inhalation of material from the skin lesions. In adults, however, chickenpox infections can be much more severe and can lead to pneumonia and birth defects in the case of infected pregnant women. Reye syndrome, mentioned earlier in this chapter, is also a serious complication associated with chickenpox, generally in children.

Once infected, most individuals acquire a lifetime immunity to future chickenpox outbreaks. For this reason, parents once held “chickenpox parties” for their children. At these events, uninfected children were intentionally exposed to an infected individual so they would contract the disease earlier in life, when the incidence of complications is very low, rather than risk a more severe infection later.

After the initial viral exposure, chickenpox has an incubation period of about 2 weeks. The initial infection of the respiratory tract leads to viremia and eventually produces fever and chills. A pustular rash then develops on the face, progresses to the trunk, and then the extremities, although most form on the trunk ([link](#)). Eventually, the lesions burst and form a crusty scab. Individuals with chickenpox are infectious from about 2 days before the outbreak of the rash until all the lesions have scabbed over.



(a)



(b)

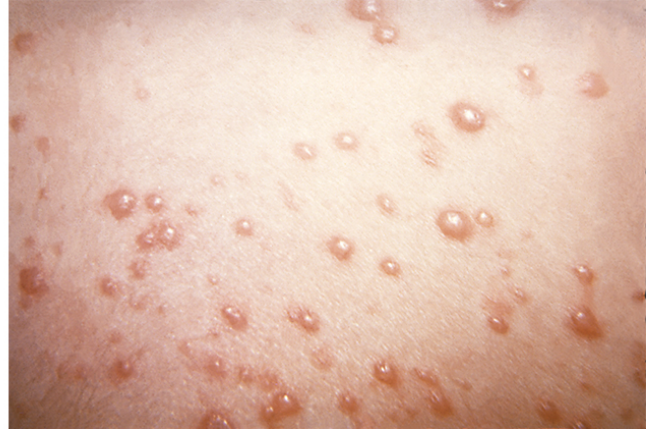
(a) The characteristic appearance of the pustular chickenpox rash is concentrated on the trunk region. (b) This transmission electron micrograph shows a viroid of human herpesvirus 3, the virus that causes chickenpox in children and shingles when it is reactivated in adults. (credit b: modification of work by Centers for Disease Control and Prevention)

Like other herpesviruses, the varicella-zoster virus can become dormant in nerve cells. While the pustular vesicles are developing, the virus moves along sensory nerves to the dorsal ganglia in the spinal cord. Once there, the varicella-zoster virus can remain latent for decades. These dormant viruses may be reactivated later in life by a variety of stimuli, including stress, aging, and immunosuppression. Once reactivated, the virus moves along sensory nerves to the skin of the face or trunk. This results in the production of the painful lesions in a condition known as **shingles** ([\[link\]](#)). These symptoms generally last for 2–6 weeks, and may recur more than once. Postherpetic neuralgia, pain signals sent from damaged nerves long after the other symptoms have subsided, is also possible. In addition, the virus can spread to other organs in immunocompromised individuals. A person with shingles lesions can transmit the virus to a nonimmune contact, and the newly infected individual would develop chickenpox as the primary infection. Shingles cannot be transmitted from one person to another.

The primary diagnosis of chickenpox in children is mainly based on the presentation of a pustular rash of the trunk. Serological and PCR-based tests are available to confirm the initial diagnosis. Treatment for chickenpox infections in children is usually not required. In patients with shingles, acyclovir treatment can often reduce the severity and length of symptoms, and diminish the risk of postherpetic neuralgia. An effective vaccine is now available for chickenpox. A vaccine is also available for adults older than 60 years who were infected with chickenpox in their youth. This vaccine reduces the likelihood of a shingles outbreak by boosting the immune defenses that are keeping the latent infection in check and preventing reactivation.



(a)



(b)

(a) An individual suffering from shingles. (b) The rash is formed because of the reactivation of a varicella-zoster infection that was initially contracted in childhood. (credit a: modification of work by National Institute of Allergy and Infectious Diseases (NIAID); credit b: modification of work by Centers for Disease Control and Prevention)

Note:

- Why does measles often lead to secondary infections?
- What signs or symptoms would distinguish rubella and measles?
- Why can chickenpox lead to shingles later in life?

Note:

Smallpox Stockpiles

Smallpox has probably killed more humans than any other infectious disease, with the possible exception of tuberculosis. This disease, caused by the variola major virus, is transmitted by inhalation of viral particles shed from lesions in the throat. The smallpox virus spreads systemically in

the bloodstream and produces a pustular skin rash. Historical epidemics of smallpox had fatality rates of 50% or greater in susceptible populations. Concerted worldwide vaccination efforts eradicated smallpox from the general population in 1977. This was the first microbial disease in history to be eradicated, a feat made possible by the fact that the only reservoir for the smallpox virus is infected humans.

Although the virus is no longer present in the wild, laboratory samples of the virus still exist in the United States and Russia.[\[footnote\]](#) The question is, why do these samples still exist? Some claim that these stocks should be maintained for research purposes. Should the smallpox virus ever reappear, they say, we would need access to such stocks for development of vaccines and treatments. Concerns about a re-emergence of the virus are not totally unfounded. Although there are no living reservoirs of the virus, there is always the possibility that smallpox could re-emerge from mummified human bodies or human remains preserved in permafrost. It is also possible that there are as-yet undiscovered samples of the virus in other locations around the world. An example of such "lost" samples was discovered in a drawer in a Food and Drug Administration lab in Maryland.[\[footnote\]](#) If an outbreak from such a source were to occur, it could lead to uncontrolled epidemics, since the population is largely unvaccinated now.

Centers for Disease Control and Prevention. "CDC Media Statement on Newly Discovered Smallpox Specimens." July 8, 2014.

<http://www.cdc.gov/media/releases/2014/s0708-nih.html>. Accessed on July 7, 2016.

Ibid.

Critics of this argument, including many research scientists and the World Health Organization, claim that there is no longer any rational argument for keeping the samples. They view the "re-emergence scenarios" as a thinly veiled pretense for harboring biological weapons. These scenarios, they say, are less probable than an intentional reintroduction of the virus from militarized stocks by humans. Furthermore, they point out that if we needed to research smallpox in the future, we could rebuild the virus from its DNA sequence.

What do you think? Are there legitimate arguments for maintaining stockpiles of smallpox, or should all forms of this deadly disease be eradicated?

Note:**Viral Infections of the Respiratory Tract**

Many viruses are capable of entering and causing disease in the respiratory system, and a number are able to spread beyond the respiratory system to cause systemic infections. Most of these infections are highly contagious and, with a few exceptions, antimicrobial drugs are not effective for treatment. Although some of these infections are self-limiting, others can have serious or fatal complications. Effective vaccines have been developed for several of these diseases, as summarized in [\[link\]](#).

Viral Infections of the Respiratory Tract				
Disease	Pathogen	Signs and Symptoms	Transmission	Vaccine
Chickenpox (varicella)	Varicella-zoster virus	In children, fever, chills, pustular rash of lesions that burst and form crusty scabs; in adults, more severe symptoms and complications (e.g., pneumonia)	Highly contagious via contact with aerosols, particles, or droplets from infected individual's blisters or respiratory secretions	Varicella (chickenpox) vaccine
Common cold	Rhinoviruses, adenoviruses, coronaviruses, others	Runny nose, congestion, sore throat, sneezing, headaches and muscle aches; may lead to otitis media, pharyngitis, laryngitis	Highly contagious via contact with respiratory secretions or inhalation of droplets or aerosols	None
Influenza	Influenza viruses A, B, C	Fever, chills, headaches, body aches, fatigue; may lead to pneumonia or complications such as Reye syndrome. Highly virulent strains may cause lethal complications	Highly contagious between humans via contact with respiratory secretions or inhalation of droplets or aerosols. Influenza A virus can be transmitted from animal reservoirs.	Vaccines developed yearly against most prevalent strains
Measles	Measles virus (MeV)	High fever, conjunctivitis, sore throat, macular rash becoming confluent, Koplik's spots on oral mucosa; in severe cases, can lead to fatal pneumonia or encephalitis, especially in children	Highly contagious via contact with respiratory secretions, skin rash, or eye secretions of infected individual	MMR
MERS	Middle East respiratory syndrome coronavirus (MERS-CoV)	Fever, cough, shortness of breath; in some cases, complications such as pneumonia and kidney failure; can be fatal	Contact with respiratory secretions or inhalation of droplets or aerosols	None
Rubella (German measles)	Rubella virus	Facial rash spreading to extremities, followed by low-grade fever, headache, conjunctivitis, cough, runny nose, swollen lymph nodes; congenital rubella may cause birth defects, miscarriage, or stillbirth	Contagious via inhalation of droplets or aerosols from infected person or asymptomatic carrier; transplacental infection from mother to fetus	MMR
SARS	SARS-associated coronavirus (SARS-CoV)	High fever, headache, body aches, dry cough, pneumonia; can be fatal	Contact with respiratory secretions or inhalation of droplets or aerosols	None
Shingles	Varicella-zoster virus	Painful lesions on face or trunk lasting several weeks; may cause postherpetic neuralgia (chronic pain) or spread to organs in severe cases	Nontransmissible; occurs when dormant virus is reactivated, generally many years after initial chicken-pox infection	Herpes zoster (shingles) vaccine
Viral pneumonia	Adenoviruses, influenza viruses, parainfluenza viruses, respiratory syncytial viruses, others	From mild cold-like symptoms to severe pneumonia; in infants, RSV infections may be life-threatening	Highly contagious via contact with respiratory secretions or inhalation of droplets or aerosols	None

Key Concepts and Summary

- Viruses cause respiratory tract infections more frequently than bacteria, and most viral infections lead to mild symptoms.
- The **common cold** can be caused by more than 200 viruses, typically rhinoviruses, coronaviruses, and adenoviruses, transmitted by direct contact, aerosols, or environmental surfaces.
- Due to its ability to rapidly mutate through **antigenic drift** and **antigenic shift**, **influenza** remains an important threat to human health. Two new influenza vaccines are developed annually.
- Several viral infections, including **respiratory syncytial virus** infections, which frequently occur in the very young, can begin with mild symptoms before progressing to viral pneumonia.
- **SARS** and **MERS** are acute respiratory infections caused by coronaviruses, and both appear to originate in animals. SARS has not been seen in the human population since 2004 but had a high mortality rate during its outbreak. MERS also has a high mortality rate and continues to appear in human populations.
- **Measles**, **rubella**, and **chickenpox** are highly contagious, systemic infections that gain entry through the respiratory system and cause rashes and fevers. Vaccines are available for all three. Measles is the most severe of the three and is responsible for significant mortality around the world. Chickenpox typically causes mild infections in children but the virus can reactivate to cause painful cases of **shingles** later in life.

Multiple Choice

Exercise:

Problem:

Which of the following viruses is not commonly associated with the common cold?

- A. coronavirus
- B. adenovirus
- C. rhinovirus
- D. varicella-zoster virus

Solution:

D

Exercise:

Problem:

Which of the following viral diseases has been eliminated from the general population worldwide?

- A. smallpox
- B. measles
- C. German measles
- D. influenza

Solution:

A

Exercise:

Problem:

What term refers to multinucleated cells that form when many host cells fuse together during infections?

- A. Ghon elements
- B. Reye syndrome
- C. Koplik's spots
- D. syncytia

Solution:

D

Exercise:

Problem:

Which of the following diseases is not associated with coronavirus infections?

- A. Middle East respiratory syndrome
- B. German measles
- C. the common cold
- D. severe acute respiratory syndrome

Solution:

B

Exercise:

Problem: Which of these viruses is responsible for causing shingles?

- A. rubella virus
- B. measles virus
- C. varicella-zoster virus
- D. variola major virus

Solution:

C

Fill in the Blank

Exercise:

Problem:

The _____ virus is responsible for causing German measles.

Solution:

rubella

Exercise:

Problem:

A(n) _____ is an uncontrolled positive feedback loop between cytokines and leucocytes.

Solution:

cytokine storm

Exercise:

Problem:

In cases of shingles, the antiviral drug _____ may be prescribed.

Solution:

acyclovir

Exercise:

Problem:

The slow accumulation of genetic changes to an influenza virus over time is referred to as _____.

Solution:

antigenic drift

Exercise:

Problem:

The _____ vaccine is effective in controlling both measles and rubella.

Solution:

MMR

Short Answer

Exercise:

Problem:

Since we all have experienced many colds in our lifetime, why are we not resistant to future infections?

Critical Thinking

Exercise:

Problem:

What role does the common cold have in the rise of antibiotic-resistant strains of bacteria in the United States?

Exercise:

Problem:

Why is it highly unlikely that influenza A virus will ever be eradicated, like the smallpox virus?

Respiratory Mycoses

LEARNING OBJECTIVES

- Identify the most common fungi that can cause infections of the respiratory tract
- Compare the major characteristics of specific fungal diseases of the respiratory tract

Fungal pathogens are ubiquitous in the environment. Serological studies have demonstrated that most people have been exposed to fungal respiratory pathogens during their lives. Yet symptomatic infections by these microbes are rare in healthy individuals. This demonstrates the efficacy of the defenses of our respiratory system. In this section, we will examine some of the fungi that can cause respiratory infections.

Histoplasmosis

Histoplasmosis is a fungal disease of the respiratory system and most commonly occurs in the Mississippi Valley of the United States and in parts of Central and South America, Africa, Asia, and Australia. The causative agent, *Histoplasma capsulatum*, is a dimorphic fungus. This microbe grows as a filamentous mold in the environment but occurs as a budding yeast during human infections. The primary reservoir for this pathogen is soil, particularly in locations rich in bat or bird feces.

Histoplasmosis is acquired by inhaling microconidial spores in the air; this disease is not transmitted from human to human. The incidence of **histoplasmosis** exposure is high in endemic areas, with 60%–90% of the

population having anti-*Histoplasma* antibodies, depending on location; [\[footnote\]](#) however, relatively few individuals exposed to the fungus actually experience symptoms. Those most likely to be affected are the very young, the elderly, and immunocompromised people.

NE Manos et al. “Geographic Variation in the Prevalence of Histoplasmin Sensitivity.” *Dis Chest* 29, no. 6 (1956):649–668.

In many ways, the course of this disease is similar to that of tuberculosis. Following inhalation, the spores enter the lungs and are phagocytized by alveolar macrophages. The fungal cells then survive and multiply within these phagocytes (see [\[link\]](#)). Focal infections cause the formation of granulomatous lesions, which can lead to calcifications that resemble the Ghon complexes of tuberculosis, even in asymptomatic cases. Also like tuberculosis, histoplasmosis can become chronic and reactivation can occur, along with dissemination to other areas of the body (e.g., the liver or spleen).

Signs and symptoms of pulmonary histoplasmosis include fever, headache, and weakness with some chest discomfort. The initial diagnosis is often based on chest radiographs and cultures grown on fungal selective media like Sabouraud's dextrose agar. Direct fluorescence antibody staining and Giemsa staining can also be used to detect this pathogen. In addition, serological tests including a complement fixation assay and histoplasmin sensitivity can be used to confirm the diagnosis. In most cases, these infections are self-limiting and antifungal therapy is not required. However, in disseminated disease, the antifungal agents amphotericin B and ketoconazole are effective; itraconazole may be effective in immunocompromised patients, in whom the disease can be more serious.

Note:

- In what environments is one more likely to be infected with histoplasmosis?
- Identify at least two similarities between histoplasmosis and tuberculosis.

Coccidioidomycosis

Infection by the dimorphic fungus *Coccidioides immitis* causes **coccidioidomycosis**. Because the microbe is endemic to the San Joaquin Valley of California, the disease is sometimes referred to as Valley fever. A related species that causes similar infections is found in semi-arid and arid regions of the southwestern United States, Mexico, and Central and South America. [\[footnote\]](#)

DR Hospenthal. “Coccidioidomycosis.” Medscape. 2015.

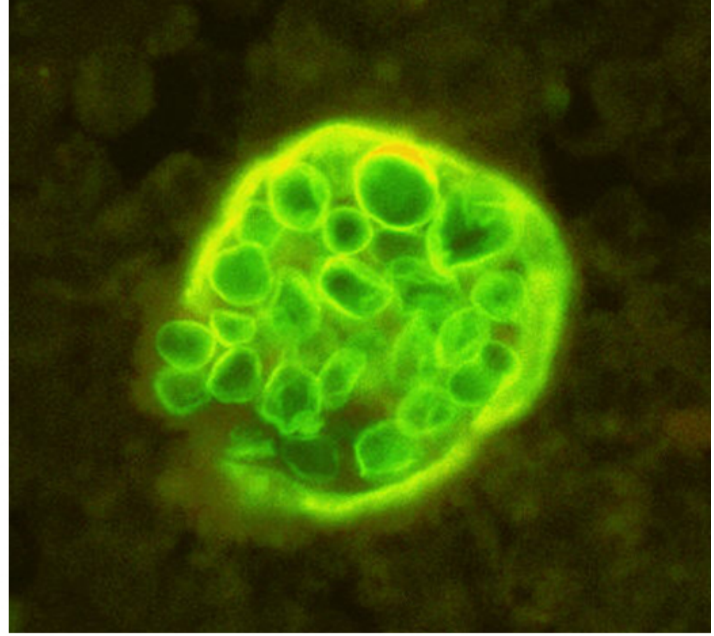
<http://emedicine.medscape.com/article/215978-overview>. Accessed July 7, 2016.

Like histoplasmosis, coccidioidomycosis is acquired by inhaling fungal spores—in this case, arthrospores formed by hyphal fragmentation. Once in the body, the fungus differentiates into spherules that are filled with endospores. Most *C. immitis* infections are asymptomatic and self-limiting. However, the infection can be very serious for immunocompromised patients. The endospores may be transported in the blood, disseminating the infection and leading to the formation of granulomatous lesions on the face and nose ([\[link\]](#)). In severe cases, other major organs can become infected, leading to serious complications such as fatal meningitis.

Coccidioidomycosis can be diagnosed by culturing clinical samples. *C. immitis* readily grows on laboratory fungal media, such as Sabouraud's dextrose agar, at 35 °C (95 °F). Culturing the fungus, however, is rather dangerous. *C. immitis* is one of the most infectious fungal pathogens known and is capable of causing laboratory-acquired infections. Indeed, until 2012, this organism was considered a “select agent” of bioterrorism and classified as a BSL-3 microbe. Serological tests for antibody production are more often used for diagnosis. Although mild cases generally do not require intervention, disseminated infections can be treated with intravenous antifungal drugs like amphotericin B.



(a)



(b)

(a) This patient has extensive facial lesions due to a disseminated *Coccidioides* infection. (b) This fluorescent micrograph depicts a spherule of *C. immitis* containing endospores. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Note:

Resolution

John's negative RIDT tests do not rule out influenza, since false-negative results are common, but the *Legionella* infection still must be treated with antibiotic therapy and is the more serious condition. John's prognosis is good, provided the physician can find an antibiotic therapy to which the infection responds.

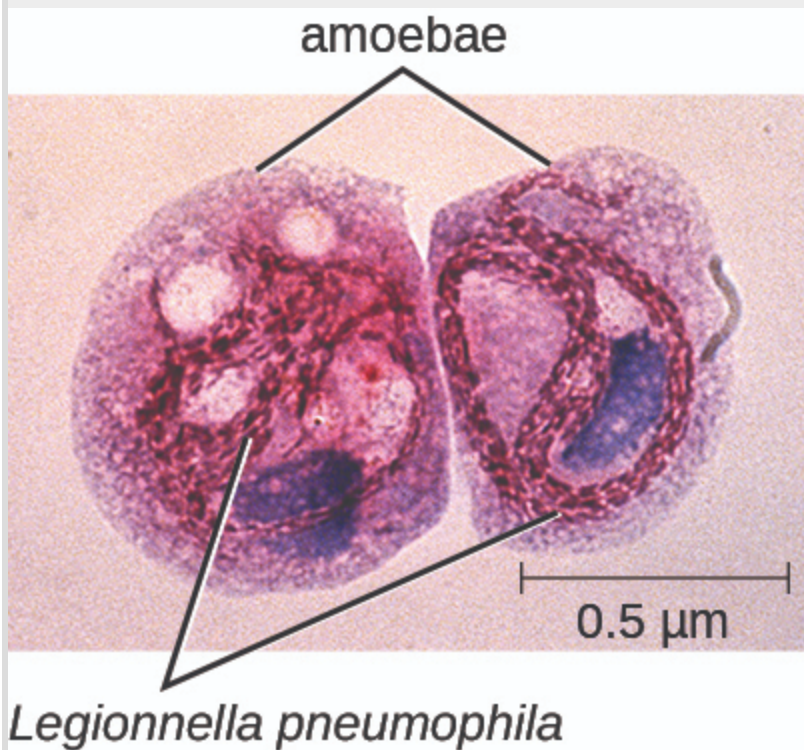
While John was undergoing treatment, three of the employees from the home improvement store also reported to the clinic with very similar symptoms. All three were older than 55 years and had *Legionella* antigen in their urine; *L. pneumophila* was also isolated from their sputum. A team from the health department was sent to the home improvement store to

identify a probable source for these infections. Their investigation revealed that about 3 weeks earlier, the store's air conditioning system, which was located where the employees ate lunch, had been undergoing maintenance. *L. pneumophila* was isolated from the cooling coils of the air conditioning system and intracellular *L. pneumophila* was observed in amoebae in samples of condensed water from the cooling coils as well ([\[link\]](#)). The amoebae provide protection for the *Legionella* bacteria and are known to enhance their pathogenicity.[\[footnote\]](#)

HY Lau and NJ Ashbolt. "The Role of Biofilms and Protozoa in *Legionella* Pathogenesis: Implications for Drinking Water." *Journal of Applied Microbiology* 107 no. 2 (2009):368–378.

In the wake of the infections, the store ordered a comprehensive cleaning of the air conditioning system and implemented a regular maintenance program to prevent the growth of biofilms within the cooling tower. They also reviewed practices at their other facilities.

After a month of rest at home, John recovered from his infection enough to return to work, as did the other three employees of the store. However, John experienced lethargy and joint pain for more than a year after his treatment.



Legionella pneumophila (red intracellular

rods) infecting amoebae from a contaminated water sample. (credit: modification of work by Centers for Disease Control and Prevention)

Go back to the [previous](#) Clinical Focus box.

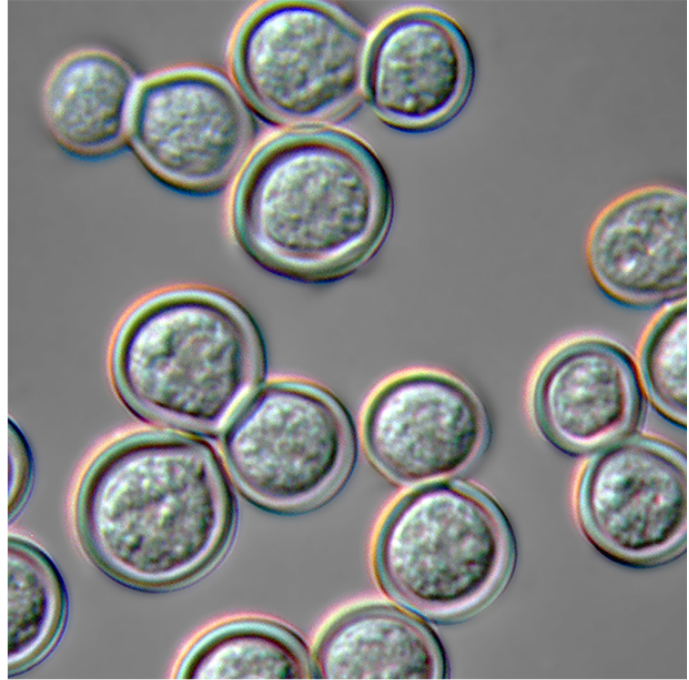
Blastomycosis

Blastomycosis is a rare disease caused by another dimorphic fungus, *Blastomyces dermatitidis*. Like *Histoplasma* and *Coccidioides*, *Blastomyces* uses the soil as a reservoir, and fungal spores can be inhaled from disturbed soil. The pulmonary form of **blastomycosis** generally causes mild flu-like symptoms and is self-limiting. It can, however, become disseminated in immunocompromised people, leading to chronic cutaneous disease with subcutaneous lesions on the face and hands ([link](#)). These skin lesions eventually become crusty and discolored and can result in deforming scars. Systemic blastomycosis is rare, but if left untreated, it is always fatal.

Preliminary diagnosis of pulmonary blastomycosis can be made by observing the characteristic budding yeast forms in sputum samples. Commercially available urine antigen tests are now also available. Additional confirmatory tests include serological assays such as immunodiffusion tests or EIA. Most cases of blastomycosis respond well to amphotericin B or ketoconazole treatments.



(a)



(b)

(a) These skin lesions are the result of disseminated cutaneous blastomycosis. (b) A differential interference contrast micrograph of *B. dermatitidis* yeast cultured on blood agar. (credit a: modification of work by Centers for Disease Control and Prevention)

Note:

Watch this [profile](#) of a blastomycosis lung infection.

Mucormycosis

A variety of fungi in the order Mucorales cause **mucormycosis**, a rare fungal disease. These include bread molds, like *Rhizopus* and *Mucor*; the most commonly associated species is *Rhizopus arrhizus (oryzae)* (see [\[link\]](#)). These fungi can colonize many different tissues in

immunocompromised patients, but often infect the skin, sinuses, or the lungs.

Although most people are regularly exposed to the causative agents of mucormycosis, infections in healthy individuals are rare. Exposure to spores from the environment typically occurs through inhalation, but the spores can also infect the skin through a wound or the gastrointestinal tract if ingested. Respiratory mucormycosis primarily affects immunocompromised individuals, such as patients with cancer or those who have had a transplant.[\[footnote\]](#)

Centers for Disease Control and Prevention. “Fungal Diseases. Definition of Mucormycosis.” 2015

<http://www.cdc.gov/fungal/diseases/mucormycosis/definition.html>.

Accessed July 7, 2016.

After the spores are inhaled, the fungi grow by extending hyphae into the host’s tissues. Infections can occur in both the upper and lower respiratory tracts. Rhinocerebral mucormycosis is an infection of the sinuses and brain; symptoms include headache, fever, facial swelling, congestion, and tissue necrosis causing black lesions in the oral cavity. Pulmonary mucormycosis is an infection of the lungs; symptoms include fever, cough, chest pain, and shortness of breath. In severe cases, infections may become disseminated and involve the central nervous system, leading to coma and death.

[\[footnote\]](#)

Centers for Disease Control and Prevention. “Fungal Diseases. Symptoms of Mucormycosis.” 2015

<http://www.cdc.gov/fungal/diseases/mucormycosis/symptoms.html>.

Accessed July 7, 2016.

Diagnosing mucormycosis can be challenging. Currently, there are no serological or PCR-based tests available to identify these infections. Tissue biopsy specimens must be examined for the presence of the fungal pathogens. The causative agents, however, are often difficult to distinguish from other filamentous fungi. Infections are typically treated by the intravenous administration of amphotericin B, and superficial infections are removed by surgical debridement. Since the patients are often immunocompromised, viral and bacterial secondary infections commonly

develop. Mortality rates vary depending on the site of the infection, the causative fungus, and other factors, but a recent study found an overall mortality rate of 54%.[\[footnote\]](#)

MM Roden et al. “Epidemiology and Outcome of Zygomycosis: A Review of 929 Reported Cases.” *Clinical Infectious Diseases* 41 no. 5 (2005):634–653.

Note:

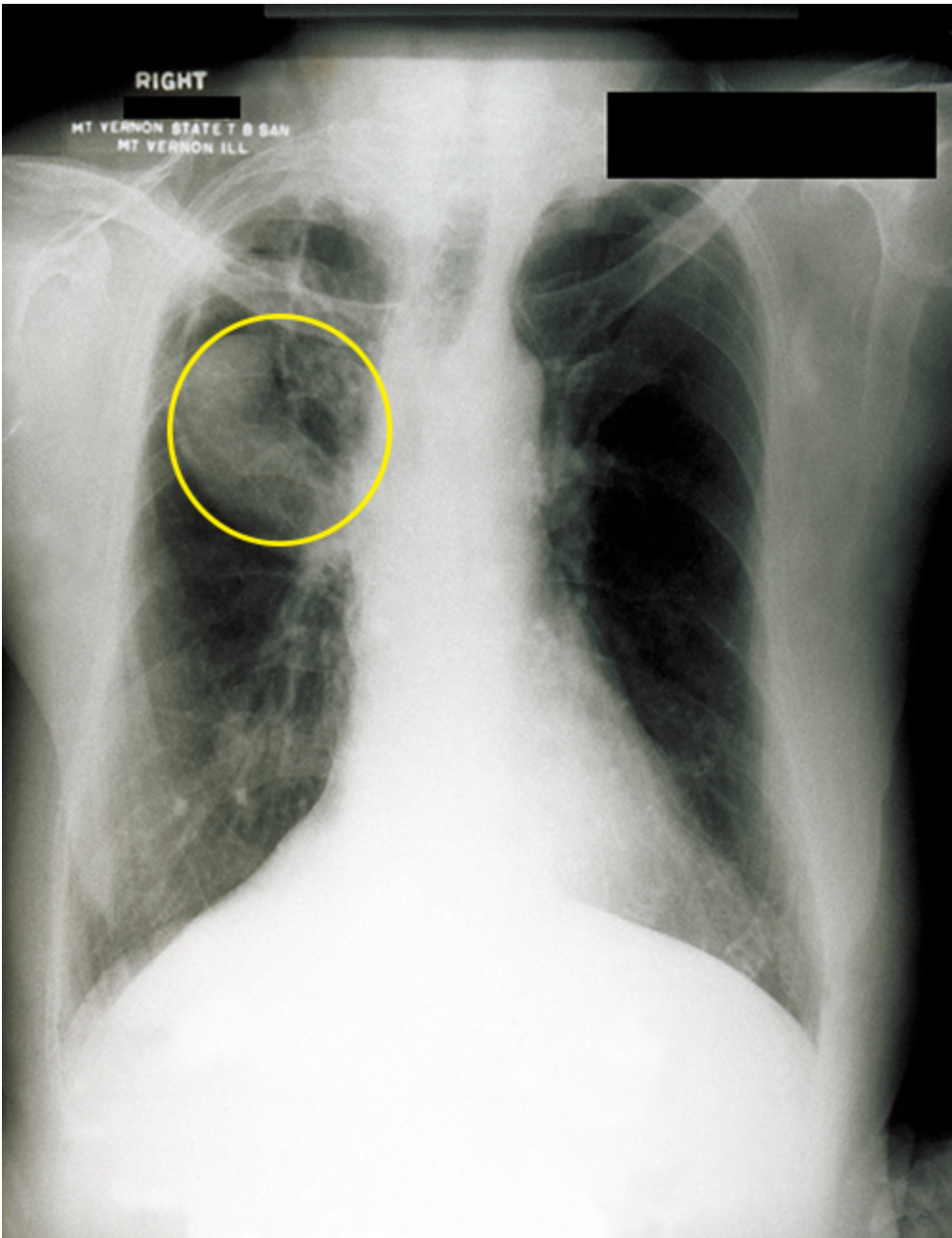
- Compare the modes of transmission for coccidioidomycosis, blastomycosis, and mucormycosis.
- In general, which are more serious: the pulmonary or disseminated forms of these infections?

Aspergillosis

Aspergillus is a common filamentous fungus found in soils and organic debris. Nearly everyone has been exposed to this mold, yet very few people become sick. In immunocompromised patients, however, *Aspergillus* may become established and cause **aspergillosis**. Inhalation of spores can lead to asthma-like allergic reactions. The symptoms commonly include shortness of breath, wheezing, coughing, runny nose, and headaches. Fungal balls, or aspergilloma, can form when hyphal colonies collect in the lungs ([\[link\]](#)). The fungal hyphae can invade the host tissues, leading to pulmonary hemorrhage and a bloody cough. In severe cases, the disease may progress to a disseminated form that is often fatal. Death most often results from pneumonia or brain hemorrhages.

Laboratory diagnosis typically requires chest radiographs and a microscopic examination of tissue and respiratory fluid samples. Serological tests are available to identify *Aspergillus* antigens. In addition, a skin test can be performed to determine if the patient has been exposed to the fungus. This test is similar to the Mantoux tuberculin skin test used for tuberculosis.

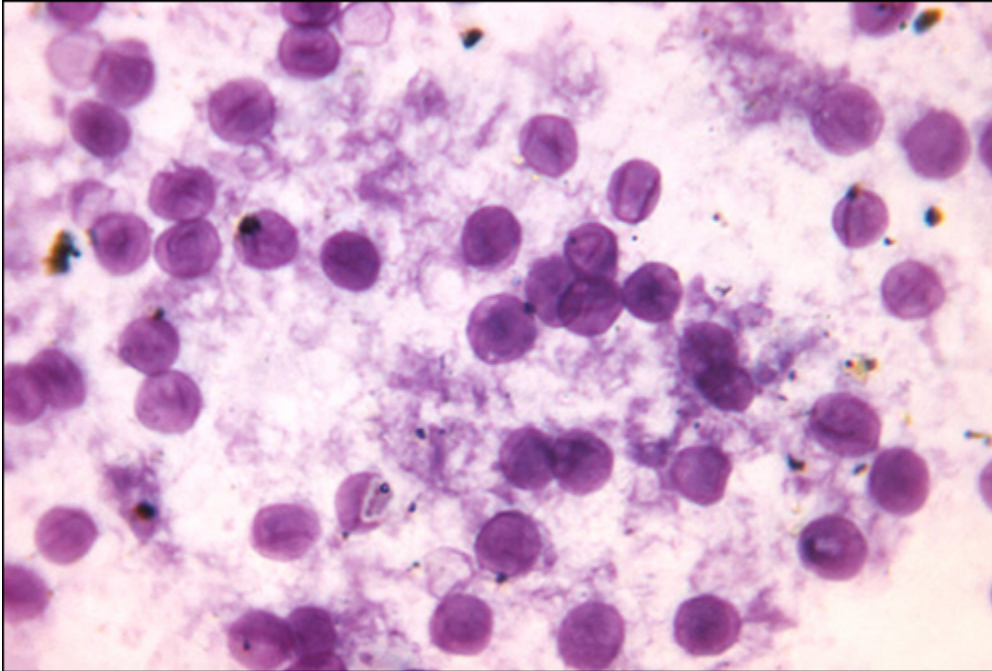
Aspergillosis is treated with intravenous antifungal agents, including itraconazole and voriconazole. Allergic symptoms can be managed with corticosteroids because these drugs suppress the immune system and reduce inflammation. However, in disseminated infections, corticosteroids must be discontinued to allow a protective immune response to occur.



A fungal ball can be observed in the upper lobe of the right lung in this chest radiograph of a patient with aspergilloma. (credit: modification of work by Centers for Disease Control and Prevention)

***Pneumocystis* Pneumonia**

A type of pneumonia called ***Pneumocystis pneumonia*** (PCP) is caused by *Pneumocystis jirovecii*. Once thought to be a protozoan, this organism was formerly named *P. carinii* but it has been reclassified as a fungus and renamed based on biochemical and genetic analyses. *Pneumocystis* is a leading cause of pneumonia in patients with acquired immunodeficiency syndrome (AIDS) and can be seen in other compromised patients and premature infants. Respiratory infection leads to fever, cough, and shortness of breath. Diagnosis of these infections can be difficult. The organism is typically identified by microscopic examination of tissue and fluid samples from the lungs ([\[link\]](#)). A PCR-based test is available to detect *P. jirovecii* in asymptomatic patients with AIDS. The best treatment for these infections is the combination drug trimethoprim-sulfamethoxazole (TMP/SMZ). These sulfa drugs often have adverse effects, but the benefits outweigh these risks. Left untreated, PCP infections are often fatal.

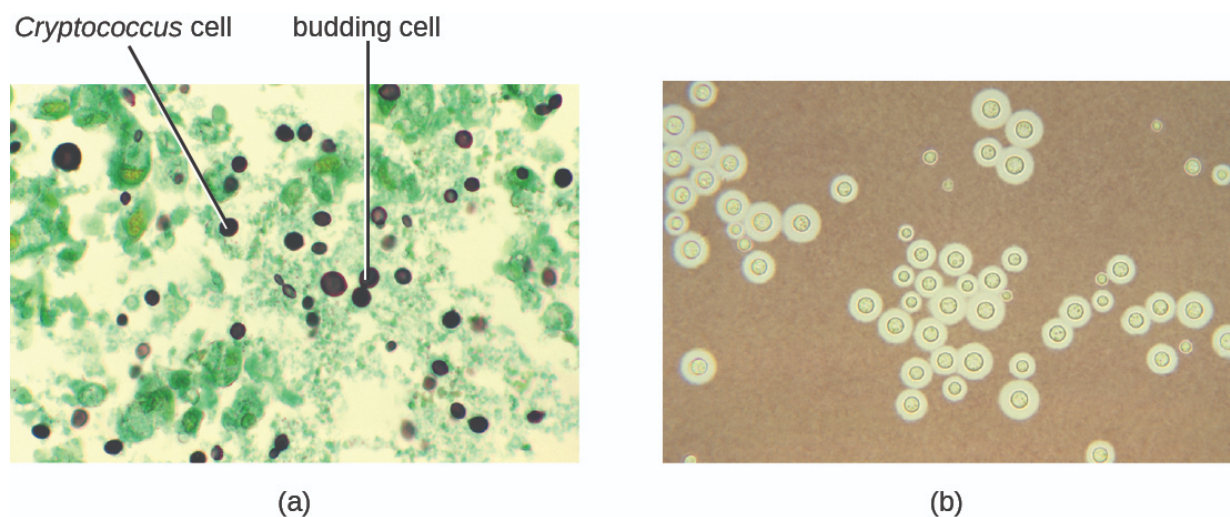


A light micrograph of a smear containing *Pneumocystis jirovecii* (dark purple cells) obtained from human lung tissue and stained with toluidine blue. (credit: Centers for Disease Control and Prevention)

Cryptococcosis

Infection by the encapsulated yeast *Cryptococcus neoformans* causes **cryptococcosis**. This fungus is ubiquitous in the soil and can be isolated from bird feces. Immunocompromised people are infected by inhaling basidiospores found in aerosols. The thick polysaccharide capsule surrounding these microbes enables them to avoid clearance by the alveolar macrophage. Initial symptoms of infection include fever, fatigue, and a dry cough. In immunocompromised patients, pulmonary infections often disseminate to the brain. The resulting meningitis produces headaches, sensitivity to light, and confusion. Left untreated, such infections are often fatal.

Cryptococcus infections are often diagnosed based on microscopic examination of lung tissues or cerebrospinal fluids. India ink preparations ([link](#)) can be used to visualize the extensive capsules that surround the yeast cells. Serological tests are also available to confirm the diagnosis. Amphotericin B, in combination with flucytosine, is typically used for the initial treatment of pulmonary infections. Amphotericin B is a broad-spectrum antifungal drug that targets fungal cell membranes. It can also adversely impact host cells and produce side effects. For this reason, clinicians must carefully balance the risks and benefits of treatments in these patients. Because it is difficult to eradicate cryptococcal infections, patients usually need to take fluconazole for up to 6 months after treatment with amphotericin B and flucytosine to clear the fungus. Cryptococcal infections are more common in immunocompromised people, such as those with AIDS. These patients typically require life-long suppressive therapy to control this fungal infection.



(a) The micrograph shows stained budding *Cryptococcus* yeast cells from the lungs of a patient with AIDS. (b) The large capsule of *Cryptococcus neoformans* is visible in this negative stain micrograph. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Note:

- What populations are most at risk for developing *Pneumocystis* pneumonia or cryptococcosis?
- Why are these infections fatal if left untreated?

Note:**Fungal Diseases of the Respiratory Tract**

Most respiratory mycoses are caused by fungi that inhabit the environment. Such infections are generally transmitted via inhalation of fungal spores and cannot be transmitted between humans. In addition, healthy people are generally not susceptible to infection even when exposed; the fungi are only virulent enough to establish infection in patients with HIV, AIDS, or another condition that compromises the immune defenses. [\[link\]](#) summarizes the features of important respiratory mycoses.

Fungal Infections of the Respiratory Tract				
Disease	Pathogen	Signs and Symptoms	Diagnostic Tests	Antimicrobial Drugs
Aspergillosis	<i>Aspergillus fumigatus</i>	Shortness of breath, wheezing, coughing, runny nose, headaches; formation of aspergillomas causing severe pneumonia and pulmonary or brain hemorrhages; can be fatal	Chest radiograph, skin test, microscopic observation of sputum samples	Itraconazole, voriconazole
Blastomycosis	<i>Blastomyces dermatitidis</i>	Fever, chills, cough, headache, fatigue, chest pain, body aches; in disseminated infections, chronic, crusted lesions on face and hands with permanent scarring; can be fatal	Microscopic observation of sputum samples; urine antigen test; EIA	Amphotericin B, ketoconazole
Coccidioidomycosis (Valley fever)	<i>Coccidioides immitis</i>	Granulomatous lesions on face and nose; may spread to organs or brain, causing fatal meningitis	Culture (in BSL-3 lab only), serological antibody tests	Amphotericin B
Cryptococcosis	<i>Cryptococcus neoformans</i>	Fever, cough, shortness of breath; can cause fatal meningitis if disseminated to brain	Microscopic examination of lung tissue or cerebrospinal fluid	Amphotericin B, fluconazole, flucytosine
Histoplasmosis	<i>Histoplasma capsulatum</i>	Fever, headache, weakness, chest pain, lesions on lungs	Chest radiograph, culture, direct fluorescence antibody staining, complement fixation assay, histoplasmin sensitivity test	Amphotericin B, ketoconazole, itraconazole
Mucormycosis	<i>Rhizopus arrhizus</i> , other <i>Rhizopus</i> spp., <i>Mucor</i> spp.	Headache, fever, facial swelling, congestion, black lesions in oral cavity, cough, chest pain, shortness of breath; often fatal	Microscopic examination of tissue biopsy specimens	Amphotericin B
<i>Pneumocystis</i> pneumonia (PCP)	<i>Pneumocystis jirovecii</i>	Fever, cough, shortness of breath; can be fatal if untreated	Microscopic examination of lung tissue and fluid, PCR	Trimethoprim-sulfamethoxazole

Key Concepts and Summary

- Fungal pathogens rarely cause respiratory disease in healthy individuals, but inhalation of fungal spores can cause severe

- pneumonia and systemic infections in immunocompromised patients.
- Antifungal drugs like amphotericin B can control most fungal respiratory infections.
 - **Histoplasmosis** is caused by a mold that grows in soil rich in bird or bat droppings. Few exposed individuals become sick, but vulnerable individuals are susceptible. The yeast-like infectious cells grow inside phagocytes.
 - **Coccidioidomycosis** is also acquired from soil and, in some individuals, will cause lesions on the face. Extreme cases may infect other organs, causing death.
 - **Blastomycosis**, a rare disease caused by a soil fungus, typically produces a mild lung infection but can become disseminated in the immunocompromised. Systemic cases are fatal if untreated.
 - **Mucormycosis** is a rare disease, caused by fungi of the order Mucorales. It primarily affects immunocompromised people. Infection involves growth of the hyphae into infected tissues and can lead to death in some cases.
 - **Aspergillosis**, caused by the common soil fungus *Aspergillus*, infects immunocompromised people. Hyphal balls may impede lung function and hyphal growth into tissues can cause damage. Disseminated forms can lead to death.
 - ***Pneumocystis pneumonia*** is caused by the fungus *P. jirovecii*. The disease is found in patients with AIDS and other immunocompromised individuals. Sulfa drug treatments have side effects, but untreated cases may be fatal.
 - **Cryptococcosis** is caused by *Cryptococcus neoformans*. Lung infections may move to the brain, causing meningitis, which can be fatal.

Multiple Choice

Exercise:

Problem: Which of these infections is also referred to as Valley fever?

- A. histoplasmosis
- B. coccidioidomycosis

- C. blastomycosis
 - D. aspergillosis
-

Solution:

B

Exercise:

Problem:

Which of the following is not caused by a dimorphic fungus?

- A. histoplasmosis
 - B. coccidioidomycosis
 - C. blastomycosis
 - D. aspergillosis
-

Solution:

D

Exercise:

Problem:

Which of the following is caused by infections by bread molds?

- A. mucormycosis
 - B. coccidioidomycosis
 - C. cryptococcosis
 - D. *Pneumocystis* pneumonia
-

Solution:

A

Exercise:

Problem: In the United States, most histoplasmosis cases occur

- A. in the Pacific northwest.
- B. in the desert southwest.
- C. in the Mississippi river valley.
- D. in Colorado river valley.

Solution:

C

Exercise:

Problem:

Which of the following infections can be diagnosed using a skin test similar to the tuberculin test?

- A. histoplasmosis
- B. cryptococcosis
- C. blastomycosis
- D. aspergillosis

Solution:

D

Fill in the Blank

Exercise:

Problem:

In coccidioidomycosis, _____ containing many endospores form in the lungs.

Solution:

spherules

Exercise:**Problem:**

In cryptococcosis, the main fungal virulence factor is the _____, which helps the pathogen avoid phagocytosis.

Solution:

capsule

Exercise:**Problem:**

In some mycoses, fungal balls called _____ form in the lungs

Solution:

aspergillomas

Exercise:

Problem: Most US cases of coccidioidomycosis occur in _____.

Solution:

the desert southwest

Exercise:**Problem:**

Coccidioidomycosis may develop when *Coccidioides immitis* _____ are inhaled.

Solution:

arthrospores

Short Answer

Exercise:

Problem:

Which pulmonary fungal infection is most likely to be confused with tuberculosis? How can we discriminate between these two types of infection?

Exercise:

Problem: Compare and contrast aspergillosis and mucormycosis.

Critical Thinking

Exercise:

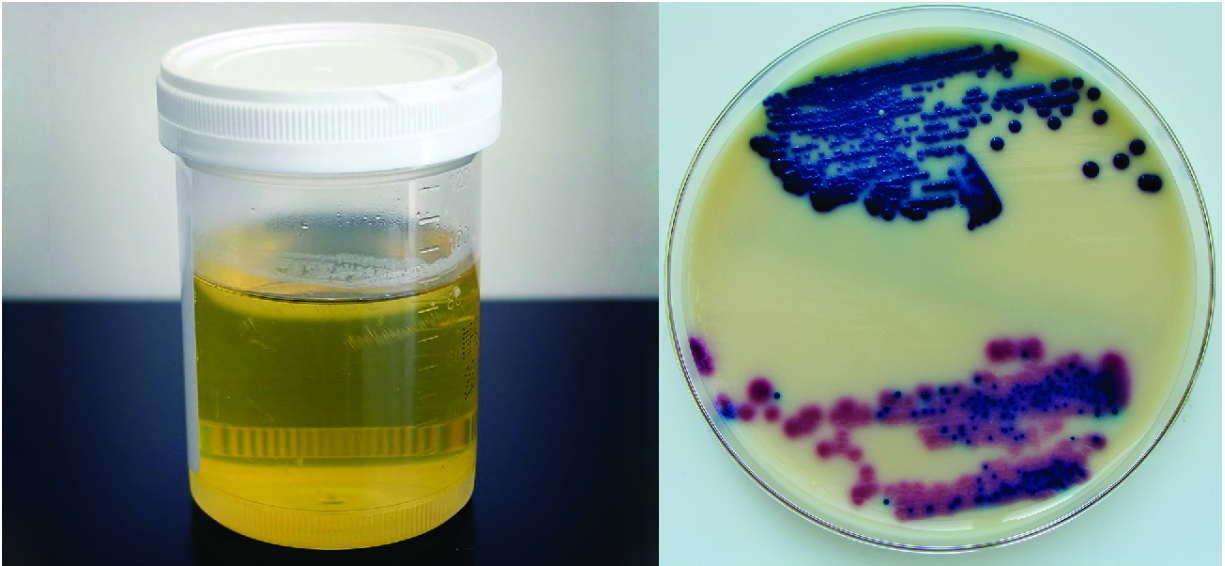
Problem:

Why are fungal pulmonary infections rarely transmissible from person to person?

Introduction

class="introduction"

Many pathogens that cause infections of the urogenital system can be detected in urine samples (left). The top sample in the culture (right) was prepared from the urine of a patient with a urinary tract infection. (credit b: modification of work by Nathan Reading)



The urogenital system is a combination of the urinary tract and reproductive system. Because both systems are open to the external environment, they are prone to infections. Some infections are introduced from outside, whereas others result from imbalances in the microbiota of the urogenital tract.

Urinary tract infections (UTIs) are one the most common bacterial infections worldwide, affecting over 100 million people each year. During 2007 in the United States, doctor office visits for UTIs exceeded 10 million, and an additional 2–3 million emergency department visits were attributed to UTIs. Sexually transmitted infections (STIs) also primarily affect the urogenital system and are an important cause of patient morbidity. The Centers for Disease Control and Prevention (CDC) estimates that there are approximately 20 million new cases of reportable STIs annually in the United States, half of which occur in people aged 15–24 years old. When STIs spread to the reproductive organs, they can be associated with severe morbidity and loss of fertility.

Because males and females have different urogenital anatomy, urogenital infections may affect males and females differently. In this chapter, we will discuss the various microbes that cause urogenital disease and the factors that contribute to their pathogenicity.

Anatomy and Normal Microbiota of the Urogenital Tract

LEARNING OBJECTIVES

- Compare the anatomy, function, and normal microbiota associated with the male and female urogenital systems
- Explain how microorganisms, in general, overcome the defenses of the urogenital system to cause infection
- Name, describe, and differentiate between general signs and symptoms associated with infections of the urogenital tract

Note:

Part 1

Nadia is a newly married 26-year-old graduate student in economics. Recently she has been experiencing an unusual vaginal discharge, as well as some itching and discomfort. Since she is due for her annual physical exam, she makes an appointment with her doctor hoping that her symptoms can be quickly treated. However, she worries that she may have some sort of sexually transmitted infection (STI). Although she is now in a monogamous relationship, she is not fully certain of her spouse's sexual history and she is reluctant to ask him about it.

At her checkup, Nadia describes her symptoms to her primary care physician and, somewhat awkwardly, explains why she thinks she might have an STI. Nadia's doctor reassures her that she regularly sees patients with similar concerns and encourages her to be fully transparent about her symptoms because some STIs can have serious complications if left untreated. After some further questioning, the doctor takes samples of Nadia's blood, urine, and vaginal discharge to be sent to the lab for testing.

- What are some possible causes of Nadia's symptoms?
- Why does the doctor take so many different samples?

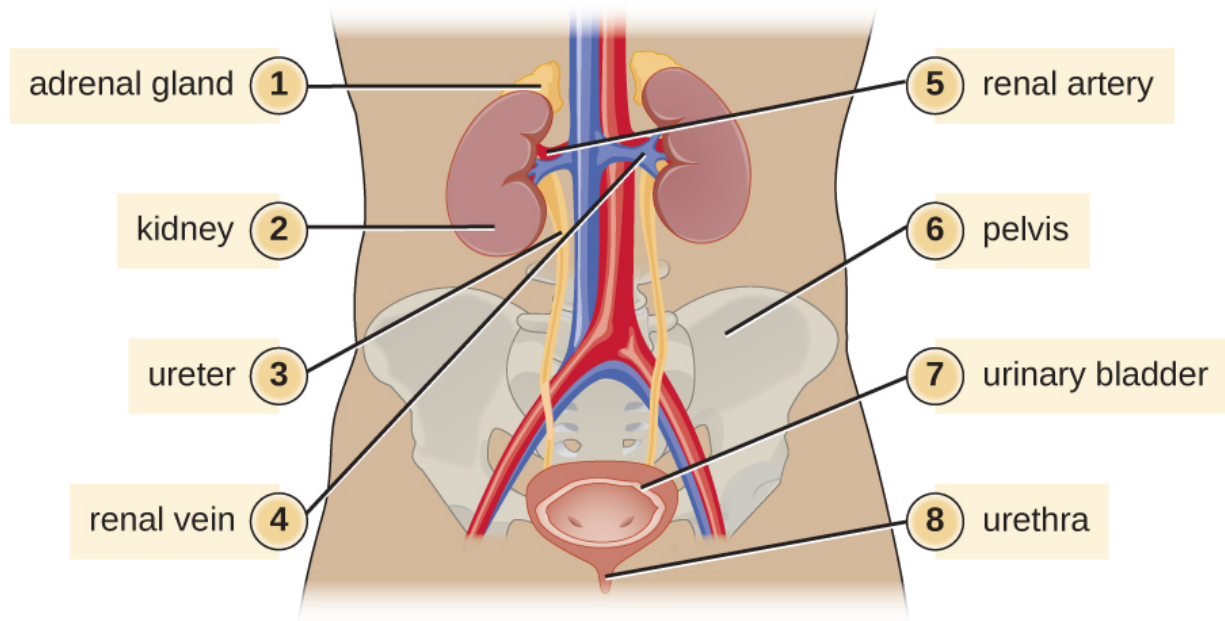
Jump to the [next](#) Clinical Focus box.

The urinary system filters blood, excretes wastes, and maintains an appropriate electrolyte and water balance. The reproductive system is responsible for the production of gametes and participates in conception and, in females, development of offspring. Due to their proximity and overlap, these systems are often studied together and referred to as the urogenital system (or genitourinary system).

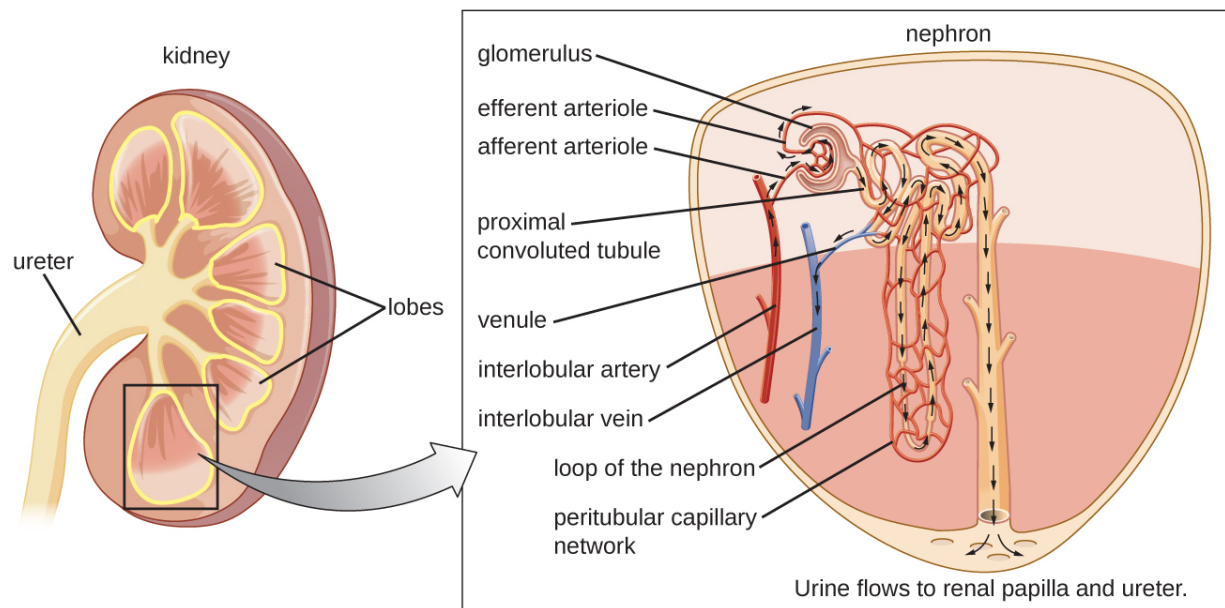
Anatomy of the Urinary Tract

The basic structures of the urinary tract are common in males and females. However, there are unique locations for these structures in females and males, and there is a significant amount of overlap between the urinary and genital structures in males. [\[link\]](#) illustrates the urinary anatomy common to females and males.

The **kidneys** carry out the urinary system's primary functions of filtering the blood and maintaining water and electrolyte balance. The kidneys are composed of millions of filtration units called nephrons. Each nephron is in intimate contact with blood through a specialized capillary bed called the **glomerulus** (plural *glomeruli*). Fluids, electrolytes, and molecules from the blood pass from the glomerulus into the nephron, creating the filtrate that becomes urine ([\[link\]](#)). Urine that collects in each kidney empties through a **ureter** and drains to the **urinary bladder**, which stores urine. Urine is released from the bladder to the **urethra**, which transports it to be excreted from the body through the **urinary meatus**, the opening of the urethra.



These structures of the human urinary system are present in both males and females.



The kidney contains several lobes, each of which contains millions of

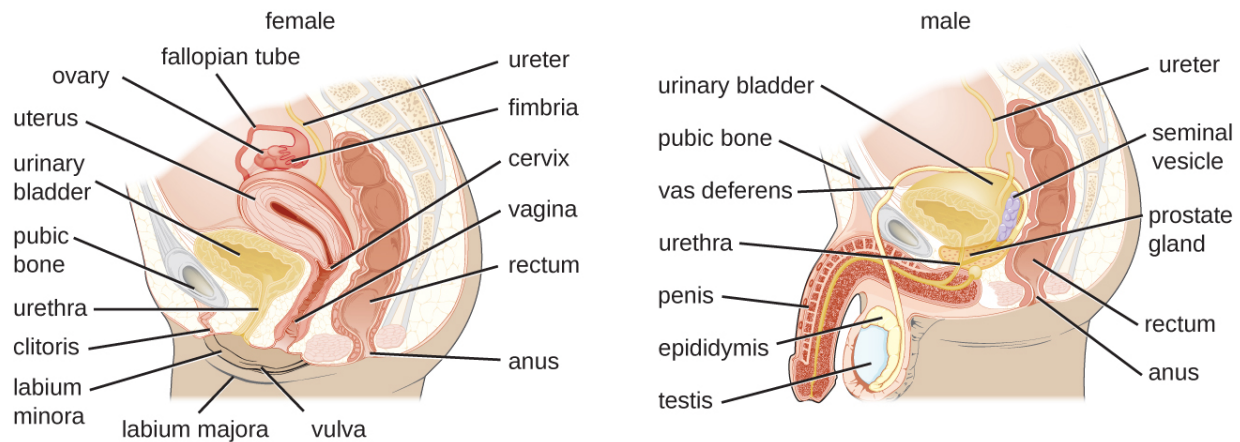
nephrons. The nephron is the functional unit of the kidney, filtering the blood and removing water and dissolved compounds. The filtrate first enters the glomerulus and then enters the proximal convoluted tubule.

As it passes through the tubule, the filtrate is further modified by osmosis and active transport until it reaches the larger ducts as urine.

Anatomy of the Reproductive System

The male reproductive system ([\[link\]](#)) is located in close proximity to the urinary system, and the urethra is part of both systems. The **testes** are responsible for the production of sperm. The **epididymis** is a coiled tube that collects sperm from the testes and passes it on to the vas deferens. The epididymis is also the site of sperm maturation after they leave the testes. The **seminal vesicles** and **prostate** are accessory glands that produce fluid that supports sperm. During ejaculation, the **vas deferens** releases this mixture of fluid and sperm, called semen, into the urethra, which extends to the end of the **penis**.

The female reproductive system is located near the urinary system ([\[link\]](#)). The external genitalia (**vulva**) in females open to the **vagina**, a muscular passageway that connects to the cervix. The **cervix** is the lower part of the **uterus** (the organ where a fertilized egg will implant and develop). The cervix is a common site of infection, especially for viruses that may lead to cervical cancer. The uterus leads to the fallopian tubes and eventually to the ovaries. Ovaries are the site of ova (egg) production, as well as the site of estrogen and progesterone production that are involved in maturation and maintenance of reproductive organs, preparation of the uterus for pregnancy, and regulation of the menstrual cycle.



The female reproductive system is located in close proximity to the urinary system. In males, the urethra is shared by the reproductive and urinary systems.

Note:

- What are the major structures of the urinary system, starting where urine is formed?
- What structure in males is shared by the reproductive and the urinary systems?

Normal Microbiota of the Urogenital System

The normal microbiota of different body sites provides an important nonspecific defense against infectious diseases (see [Physical Defenses](#)), and the urogenital tract is no exception. In both men and women, however, the kidneys are sterile. Although urine does contain some antibacterial components, bacteria will grow in urine left out at room temperature. Therefore, it is primarily the flushing action that keeps the ureters and bladder free of microbes.

Below the bladder, the normal microbiota of the male urogenital system is found primarily within the distal urethra and includes bacterial species that are commonly associated with the skin microbiota. In women, the normal microbiota is found within the distal one third of the urethra and the vagina. The normal microbiota of the vagina becomes established shortly after birth and is a complex and dynamic population of bacteria that fluctuates in response to environmental changes. Members of the vaginal microbiota play an important role in the nonspecific defense against vaginal infections and sexually transmitted infections by occupying cellular binding sites and competing for nutrients. In addition, the production of lactic acid by members of the microbiota provides an acidic environment within the vagina that also serves as a defense against infections. For the majority of women, the lactic-acid-producing bacteria in the vagina are dominated by a variety of species of *Lactobacillus*. For women who lack sufficient lactobacilli in their vagina, lactic acid production comes primarily from other species of bacteria such as *Leptotrichia* spp., *Megasphaera* spp., and *Atopobium vaginae*. *Lactobacillus* spp. use glycogen from vaginal epithelial cells for metabolism and production of lactic acid. This process is tightly regulated by the hormone estrogen. Increased levels of estrogen correlate with increased levels of vaginal glycogen, increased production of lactic acid, and a lower vaginal pH. Therefore, decreases in estrogen during the menstrual cycle and with menopause are associated with decreased levels of vaginal glycogen and lactic acid, and a higher pH. In addition to producing lactic acid, *Lactobacillus* spp. also contribute to the defenses against infectious disease through their production of hydrogen peroxide and bacteriocins (antibacterial peptides).

Note:

- What factors affect the microbiota of the female reproductive tract?

General Signs and Symptoms of Urogenital Infections

Infections of the urinary tract most commonly cause inflammation of the bladder (**cystitis**) or of the urethra (**urethritis**). Urethritis can be associated with cystitis, but can also be caused by sexually transmitted infections. Symptoms of urethritis in men include burning sensation while urinating, discharge from the penis, and blood in the semen or the urine. In women, urethritis is associated with painful and frequent urination, vaginal discharge, fever, chills, and abdominal pain. The symptoms of cystitis are similar to those of urethritis. When urethritis is caused by a sexually transmitted pathogen, additional symptoms involving the genitalia can occur. These can include painful vesicles (blisters), warts, and ulcers. Ureteritis, a rare infection of the ureter, can also occur with cystitis. These infections can be acute or chronic.

Pyelonephritis and **glomerulonephritis** are infections of the kidney that are potentially serious. Pyelonephritis is an infection of one or both of the kidneys and may develop from a lower urinary tract infection; the upper urinary tract, including the ureters, is often affected. Signs and symptoms of pyelonephritis include fever, chills, nausea, vomiting, lower back pain, and frequent painful urination. Pyelonephritis usually only becomes chronic in individuals who have malformations in or damage to the kidneys.

Glomerulonephritis is an inflammation of the glomeruli of the nephrons. Symptoms include excessive protein and blood in urine, increased blood pressure, and fluid retention leading to edema of face, hands, and feet. Glomerulonephritis may be an acute infection or it can become chronic.

Infections occurring within the reproductive structures of males include epididymitis, orchitis, and prostatitis. Bacterial infections may cause inflammation of the epididymis, called **epididymitis**. This inflammation causes pain in the scrotum, testicles, and groin; swelling, redness, and warm skin in these areas may also be observed. Inflammation of the testicle, called **orchitis**, is usually caused by a bacterial infection spreading from the epididymis, but it can also be a complication of mumps, a viral disease. The symptoms are similar to those of epididymitis, and it is not uncommon for them both to occur together, in which case the condition is called epididymo-orchitis. Inflammation of the prostate gland, called **prostatitis**, can result from a bacterial infection. The signs and symptoms of prostatitis

include fever, chills, and pain in the bladder, testicles, and penis. Patients may also experience burning during urination, difficulty emptying the bladder, and painful ejaculation.

Because of its proximity to the exterior, the vagina is a common site for infections in women. The general term for any inflammation of the vagina is **vaginitis**. Vaginitis often develops as a result of an overgrowth of bacteria or fungi that normally reside in the vaginal microbiota, although it can also result from infections by transient pathogens. Bacterial infections of the vagina are called bacterial **vaginosis**, whereas fungal infections (typically involving *Candida* spp.) are called **yeast infections**. Dynamic changes affecting the normal microbiota, acid production, and pH variations can be involved in the initiation of the microbial overgrowth and the development of vaginitis. Although some individuals may have no symptoms, vaginosis and vaginitis can be associated with discharge, odor, itching, and burning.

Pelvic inflammatory disease (PID) is an infection of the female reproductive organs including the uterus, cervix, fallopian tubes, and ovaries. The two most common pathogens are the sexually transmitted bacterial pathogens *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Inflammation of the fallopian tubes, called **salpingitis**, is the most serious form of PID. Symptoms of PID can vary between women and include pain in the lower abdomen, vaginal discharge, fever, chills, nausea, diarrhea, vomiting, and painful urination.

Note:

- What conditions can result from infections affecting the urinary system?
- What are some common causes of vaginitis in women?

General Causes and Modes of Transmission of Urogenital Infections

Hormonal changes, particularly shifts in estrogen in women due to pregnancy or menopause, can increase susceptibility to urogenital infections. As discussed earlier, estrogen plays an important role in regulating the availability of glycogen and subsequent production of lactic acid by *Lactobacillus* species. Low levels of estrogen are associated with an increased vaginal pH and an increased risk of bacterial vaginosis and yeast infections. Estrogen also plays a role in maintaining the elasticity, strength, and thickness of the vaginal wall, and keeps the vaginal wall lubricated, reducing dryness. Low levels of estrogen are associated with thinning of the vaginal wall. This thinning increases the risk of tears and abrasions, which compromise the protective barrier and increase susceptibility to pathogens.

Another common cause of urogenital infections in females is fecal contamination that occurs because of the close proximity of the anus and the urethra. *Escherichia coli*, an important member of the digestive tract microbiota, is the most common cause of urinary tract infections (urethritis and cystitis) in women; it generally causes infection when it is introduced to the urethra in fecal matter. Good hygiene can reduce the risk of urinary tract infections by this route. In men, urinary tract infections are more commonly associated with other conditions, such as an enlarged prostate, kidney stones, or placement of a urinary catheter. All of these conditions impair the normal emptying of the bladder, which serves to flush out microbes capable of causing infection.

Infections that are transmitted between individuals through sexual contact are called sexually transmitted infections (STIs) or sexually transmitted diseases (STDs). (The CDC prefers the term STD, but WHO prefers STI, [\[footnote\]](#) which encompasses infections that result in disease as well as those that are subclinical or asymptomatic.) STIs often affect the external genitalia and skin, where microbes are easily transferred through physical contact. Lymph nodes in the genital region may also become swollen as a result of infection. However, many STIs have systemic effects as well, causing symptoms that range from mild (e.g., general malaise) to severe (e.g., liver damage or serious immunosuppression).

World Health Organization. "Guidelines for the Management of Sexually Transmitted Infections." World Health Organization, 2003.
<http://www.who.int/hiv/pub/sti/en/STIGuidelines2003.pdf>.

Note:

- What role does *Lactobacillus* play in the health of the female reproductive system?
- Why do urinary tract infections have different causes in males and females?

Key Concepts and Summary

- The urinary system is responsible for filtering the blood, excreting wastes, and helping to regulate electrolyte and water balance.
- The urinary system includes the **kidneys, ureters, urinary bladder, and urethra**; the bladder and urethra are the most common sites of infection.
- Common sites of infection in the male reproductive system include the urethra, as well as the testes, **prostate** and **epididymis**.
- The most common sites of infection in the female reproductive system are the **vulva, vagina, cervix, and fallopian tubes**.
- Infections of the urogenital tract can occur through colonization from the external environment, alterations in microbiota due to hormonal or other physiological and environmental changes, fecal contamination, and sexual transmission (STIs).

Multiple Choice

Exercise:

Problem: When it first leaves the kidney, urine flows through

- a. the urinary bladder.
 - b. the urethra.
 - c. the ureter.
 - d. the glomeruli.
-

Solution:

C

Exercise:

Problem:

What part of the male urogenital tract is shared by the urinary and reproductive systems?

- a. the prostate gland
 - b. the seminal vesicles
 - c. the vas deferens
 - d. the urethra
-

Solution:

D

Fill in the Blank

Exercise:

Problem:

The genus of bacteria found in the vagina that is important in maintaining a healthy environment, including an acidic pH, is _____.

Solution:

Lactobacillus

Short Answer

Exercise:

Problem:

When the microbial balance of the vagina is disrupted, leading to overgrowth of resident bacteria without necessarily causing inflammation, the condition is called _____.

Solution:

vaginosis

Exercise:

Problem:

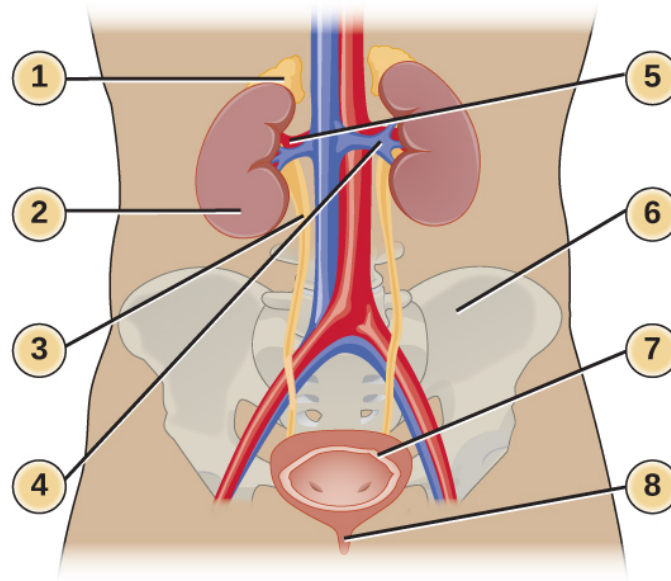
Explain the difference between a sexually transmitted infection and a sexually transmitted disease.

Solution:

An STI is a broader term, including colonization by organisms that may not necessarily cause disease.

Exercise:

Problem: In the figure shown here, where would cystitis occur?



Critical Thinking

Exercise:

Problem:

Epidemiological data show that the use of antibiotics is often followed by cases of vaginosis or vaginitis in women. Can you explain this finding?

Bacterial Infections of the Urinary System

LEARNING OBJECTIVES

- Identify the most common bacterial pathogens that can cause urinary tract infections
- Compare the major characteristics of specific bacterial diseases affecting the urinary tract

Urinary tract infections (UTIs) include infections of the urethra, bladder, and kidneys, and are common causes of urethritis, cystitis, pyelonephritis, and glomerulonephritis. Bacteria are the most common causes of UTIs, especially in the urethra and bladder.

Cystitis

Cystitis is most often caused by a bacterial infection of the bladder, but it can also occur as a reaction to certain treatments or irritants such as radiation treatment, hygiene sprays, or spermicides. Common symptoms of cystitis include **dysuria** (urination accompanied by burning, discomfort, or pain), **pyuria** (pus in the urine), **hematuria** (blood in the urine), and bladder pain.

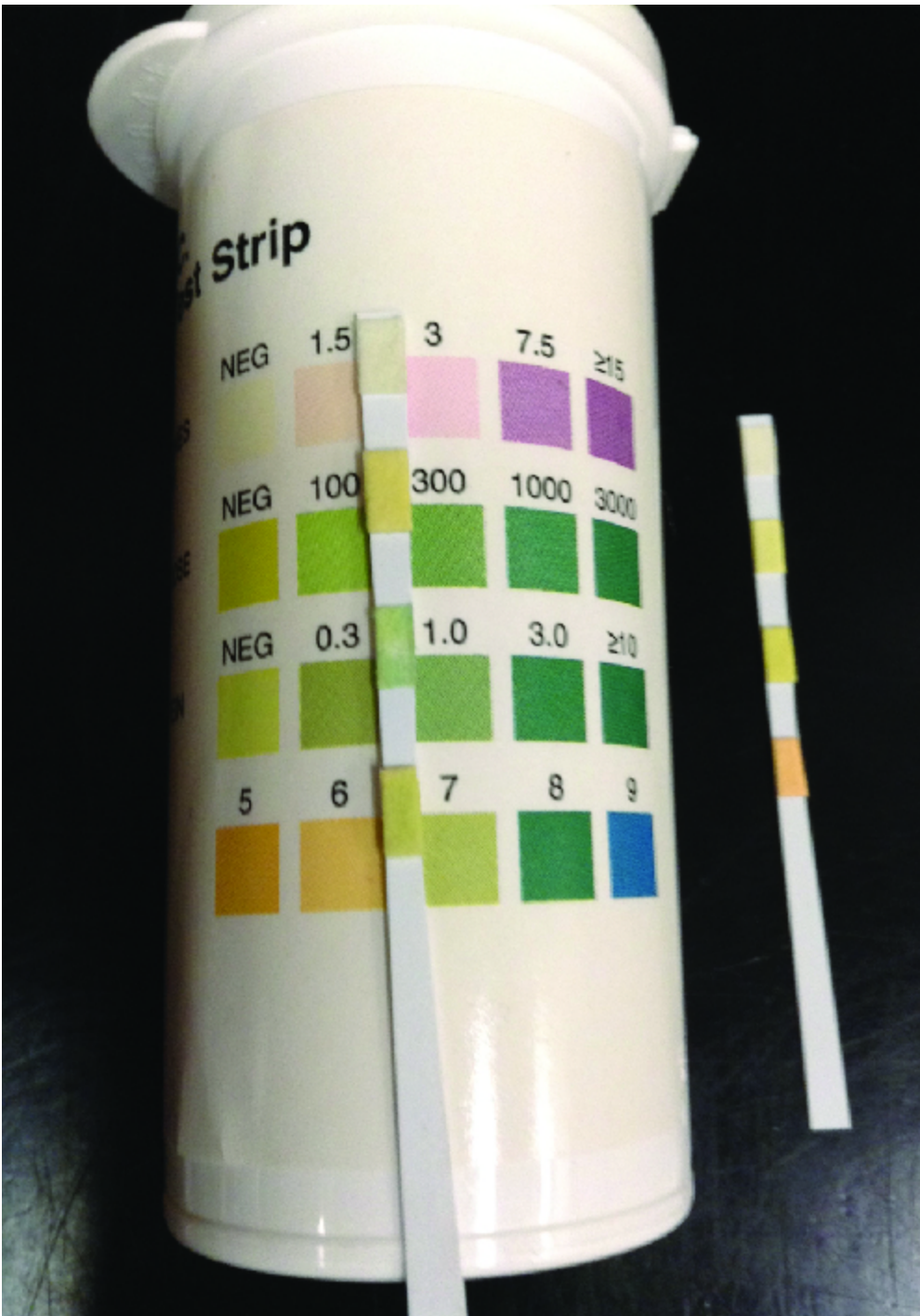
In women, bladder infections are more common because the urethra is short and located in close proximity to the anus, which can result in infections of the urinary tract by fecal bacteria. Bladder infections are also more common in the elderly because the bladder may not empty fully, causing urine to pool; the elderly may also have weaker immune systems that make them more vulnerable to infection. Conditions such as prostatitis in men or

kidney stones in both men and women can impact proper drainage of urine and increase risk of bladder infections. Catheterization can also increase the risk of bladder infection (see [Case in Point: Cystitis in the Elderly](#)).

Gram-negative bacteria such as *Escherichia coli* (most commonly), *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* cause most bladder infections. Gram-positive pathogens associated with cystitis include the coagulase-negative *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Streptococcus agalactiae*. Routine manual urinalysis using a urine dipstick or test strip can be used for rapid screening of infection. These test strips ([link](#)) are either held in a urine stream or dipped in a sample of urine to test for the presence of nitrites, leukocyte esterase, protein, or blood that can indicate an active bacterial infection. The presence of nitrite may indicate the presence of *E. coli* or *K. pneumoniae*; these bacteria produce nitrate reductase, which converts nitrate to nitrite. The leukocyte esterase (LE) test detects the presence of neutrophils as an indication of active infection.

Low specificity, sensitivity, or both, associated with these rapid screening tests require that care be taken in interpretation of results and in their use in diagnosis of urinary tract infections. Therefore, positive LE or nitrite results are followed by a urine culture to confirm a bladder infection. Urine culture is generally accomplished using blood agar and MacConkey agar, and it is important to culture a clean catch of urine to minimize contamination with normal microbiota of the penis and vagina. A clean catch of urine is accomplished by first washing the labia and urethral opening of female patients or the penis of male patients. The patient then releases a small amount of urine into the toilet bowl before stopping the flow of urine. Finally, the patient resumes urination, this time filling the container used to collect the specimen.

Bacterial cystitis is commonly treated with fluoroquinolones, nitrofurantoin, cephalosporins, or a combination of trimethoprim and sulfamethoxazole. Pain medications may provide relief for patients with dysuria. Treatment is more difficult in elderly patients, who experience a higher rate of complications such as sepsis and kidney infections.



A urine dipstick is compared against a color key to determine levels of various chemicals, proteins, or cells in the urine. Abnormal levels may indicate an infection.
(credit: modification of work by Suzanne Wakim)

Note:**Cystitis in the Elderly**

Robert, an 81-year-old widower with early onset Alzheimer's, was recently moved to a nursing home because he was having difficulty living on his own. Within a few weeks of his arrival, he developed a fever and began to experience pain associated with urination. He also began having episodes of confusion and delirium. The doctor assigned to examine Robert read his file and noticed that Robert was treated for prostatitis several years earlier. When he asked Robert how often he had been urinating, Robert explained that he had been trying not to drink too much so that he didn't have to walk to the restroom.

All of this evidence suggests that Robert likely has a urinary tract infection. Robert's age means that his immune system has probably begun to weaken, and his previous prostate condition may be making it difficult for him to empty his bladder. In addition, Robert's avoidance of fluids has led to dehydration and infrequent urination, which may have allowed an infection to establish itself in his urinary tract. The fever and dysuria are common signs of a UTI in patients of all ages, and UTIs in elderly patients are often accompanied by a notable decline in mental function.

Physical challenges often discourage elderly individuals from urinating as frequently as they would otherwise. In addition, neurological conditions that disproportionately affect the elderly (e.g., Alzheimer's and Parkinson's disease) may also reduce their ability to empty their bladders. Robert's doctor noted that he was having difficulty navigating his new home and recommended that he be given more assistance and that his fluid intake be monitored. The doctor also took a urine sample and ordered a laboratory culture to confirm the identity of the causative agent.

- Why is it important to identify the causative agent in a UTI?
- Should the doctor prescribe a broad-spectrum or narrow-spectrum antibiotic to treat Robert's UTI? Why?

Kidney Infections (Pyelonephritis and Glomerulonephritis)

Pyelonephritis, an inflammation of the kidney, can be caused by bacteria that have spread from other parts of the urinary tract (such as the bladder). In addition, pyelonephritis can develop from bacteria that travel through the bloodstream to the kidney. When the infection spreads from the lower urinary tract, the causative agents are typically fecal bacteria such as *E. coli*. Common signs and symptoms include back pain (due to the location of the kidneys), fever, and nausea or vomiting. Gross hematuria (visible blood in the urine) occurs in 30–40% of women but is rare in men.^[footnote] The infection can become serious, potentially leading to bacteremia and systemic effects that can become life-threatening. Scarring of the kidney can occur and persist after the infection has cleared, which may lead to dysfunction.

Tibor Fulop. “Acute Pyelonephritis” *Medscape*, 2015.

<http://emedicine.medscape.com/article/245559-overview>.

Diagnosis of pyelonephritis is made using microscopic examination of urine, culture of urine, testing for leukocyte esterase and nitrite levels, and examination of the urine for blood or protein. It is also important to use blood cultures to evaluate the spread of the pathogen into the bloodstream. Imaging of the kidneys may be performed in high-risk patients with diabetes or immunosuppression, the elderly, patients with previous renal damage, or to rule out an obstruction in the kidney. Pyelonephritis can be treated with either oral or intravenous antibiotics, including penicillins, cephalosporins, vancomycin, fluoroquinolones, carbapenems, and aminoglycosides.

Glomerulonephritis occurs when the glomeruli of the nephrons are damaged from inflammation. Whereas pyelonephritis is usually acute, glomerulonephritis may be acute or chronic. The most well-characterized mechanism of glomerulonephritis is the post-streptococcal sequelae associated with *Streptococcus pyogenes* throat and skin infections. Although *S. pyogenes* does not directly infect the glomeruli of the kidney, immune complexes that form in blood between *S. pyogenes* antigens and antibodies lodge in the capillary endothelial cell junctions of the glomeruli and trigger a damaging inflammatory response. Glomerulonephritis can also

occur in patients with bacterial endocarditis (infection and inflammation of heart tissue); however, it is currently unknown whether glomerulonephritis associated with endocarditis is also immune-mediated.

Leptospirosis

Leptospira are generally harmless spirochetes that are commonly found in the soil. However, some pathogenic species can cause an infection called **leptospirosis** in the kidneys and other organs ([\[link\]](#)). Leptospirosis can produce fever, headache, chills, vomiting, diarrhea, and rash with severe muscular pain. If the disease continues to progress, infection of the kidney, meninges, or liver may occur and may lead to organ failure or meningitis. When the kidney and liver become seriously infected, it is called **Weil's disease**. Pulmonary hemorrhagic syndrome can also develop in the lungs, and jaundice may occur.

Leptospira spp. are found widely in animals such as dogs, horses, cattle, pigs, and rodents, and are excreted in their urine. Humans generally become infected by coming in contact with contaminated soil or water, often while swimming or during flooding; infection can also occur through contact with body fluids containing the bacteria. The bacteria may enter the body through mucous membranes, skin injuries, or by ingestion. The mechanism of pathogenicity is not well understood.

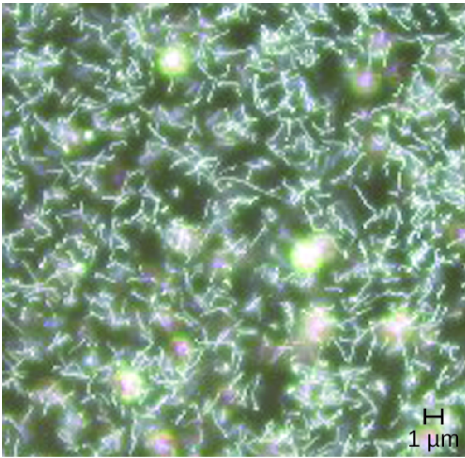
Leptospirosis is extremely rare in the United States, although it is endemic in Hawaii; 50% of all cases in the United States come from Hawaii.

[\[footnote\]](#) It is more common in tropical than in temperate climates, and individuals who work with animals or animal products are most at risk. The bacteria can also be cultivated in specialized media, with growth observed in broth in a few days to four weeks; however, diagnosis of leptospirosis is generally made using faster methods, such as detection of antibodies to *Leptospira* spp. in patient samples using serologic testing. Polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), slide agglutination, and indirect immunofluorescence tests may all be used for diagnosis. Treatment for leptospirosis involves broad-spectrum antibiotics

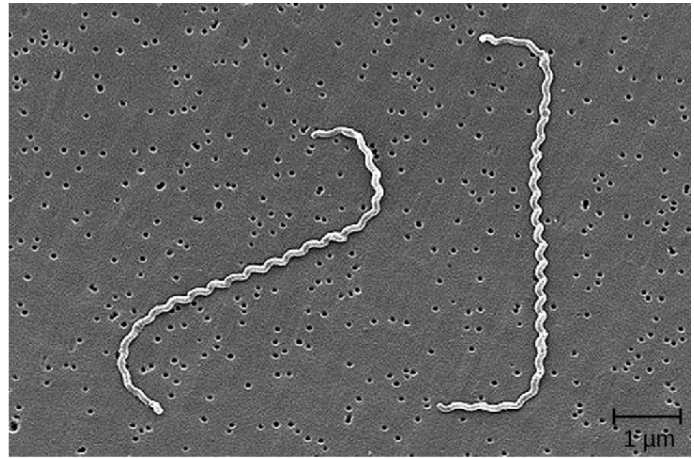
such as penicillin and doxycycline. For more serious cases of leptospirosis, antibiotics may be given intravenously.

Centers for Disease Control and Prevention. "Leptospirosis." 2015.

http://www.cdc.gov/leptospirosis/health_care_workers.



(a)



(b)

(a) Dark field view of *Leptospira* sp. (b) A scanning electron micrograph of *Leptospira interrogans*, a pathogenic species, shows the distinctive spirochete morphology of this genus. (credit b: modification of work by Janice Carr, Centers for Disease Control and Prevention)

Note:

- What is the most common cause of a kidney infection?
- What are the most common symptoms of a kidney infection?

Nongonococcal Urethritis (NGU)

There are two main categories of bacterial urethritis: gonorrheal and nongonococcal. Gonorrheal urethritis is caused by *Neisseria gonorrhoeae* and is associated with gonorrhea, a common STI. This cause of urethritis will be discussed in [Bacterial Infections of the Reproductive System](#). The term **nongonococcal urethritis (NGU)** refers to inflammation of the urethra that is unrelated to *N. gonorrhoeae*. In women, NGU is often asymptomatic. In men, NGU is typically a mild disease, but can lead to purulent discharge and dysuria. Because the symptoms are often mild or nonexistent, most infected individuals do not know that they are infected, yet they are carriers of the disease. Asymptomatic patients also have no reason to seek treatment, and although not common, untreated NGU can spread to the reproductive organs, causing pelvic inflammatory disease and salpingitis in women and epididymitis and prostatitis in men. Important bacterial pathogens that cause nongonococcal urethritis include *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*.

C. trachomatis is a difficult-to-stain, gram-negative bacterium with an ovoid shape. An intracellular pathogen, *C. trachomatis* causes the most frequently reported STI in the United States, chlamydia. Although most persons infected with *C. trachomatis* are asymptomatic, some patients can present with NGU. *C. trachomatis* can also cause non-urogenital infections such as the ocular disease trachoma (see [Bacterial Infections of the Skin and Eyes](#)). The life cycle of *C. trachomatis* is illustrated in [\[link\]](#).

C. trachomatis has multiple possible virulence factors that are currently being studied to evaluate their roles in causing disease. These include polymorphic outer-membrane autotransporter proteins, stress response proteins, and type III secretion effectors. The type III secretion effectors have been identified in gram-negative pathogens, including *C. trachomatis*. This virulence factor is an assembly of more than 20 proteins that form what is called an injectisome for the transfer of other effector proteins that target the infected host cells. The outer-membrane autotransporter proteins are also an effective mechanism of delivering virulence factors involved in colonization, disease progression, and immune system evasion.

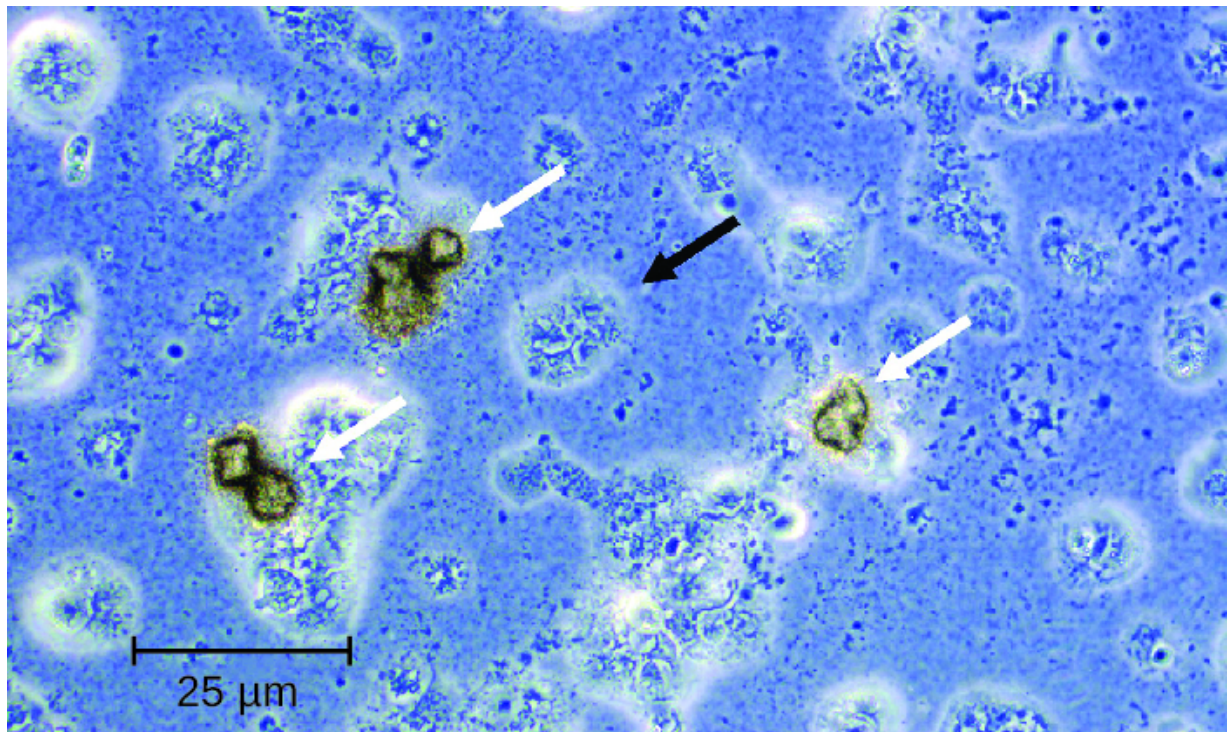
Other species associated with NGU include *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*. These bacteria are commonly found in the normal microbiota of healthy individuals, who may acquire them during birth or through sexual contact, but they can sometimes cause infections leading to urethritis (in males and females) or vaginitis and cervicitis (in females).

M. genitalium is a more common cause of urethritis in most settings than *N. gonorrhoeae*, although it is less common than *C. trachomatis*. It is responsible for approximately 30% of recurrent or persistent infections, 20–25% of nonchlamydial NGU cases, and 15%–20% of NGU cases. *M. genitalium* attaches to epithelial cells and has substantial antigenic variation that helps it evade host immune responses. It has lipid-associated membrane proteins that are involved in causing inflammation.

Several possible virulence factors have been implicated in the pathogenesis of *U. urealyticum* ([\[link\]](#)). These include the ureaplasma proteins phospholipase A, phospholipase C, multiple banded antigen (MBA), urease, and immunoglobulin α protease. The phospholipases are virulence factors that damage the cytoplasmic membrane of target cells. The immunoglobulin α protease is an important defense against antibodies. It can generate hydrogen peroxide, which may adversely affect host cell membranes through the production of reactive oxygen species.

Treatments differ for gonorrheal and nongonococcal urethritis. However, *N. gonorrhoeae* and *C. trachomatis* are often simultaneously present, which is an important consideration for treatment. NGU is most commonly treated using tetracyclines (such as doxycycline) and azithromycin; erythromycin is an alternative option. Tetracyclines and fluoroquinolones are most commonly used to treat *U. urealyticum*, but resistance to tetracyclines is becoming an increasing problem.[\[footnote\]](#) While tetracyclines have been the treatment of choice for *M. hominis*, increasing resistance means that other options must be used. Clindamycin and fluoroquinolones are alternatives. *M. genitalium* is generally susceptible to doxycycline, azithromycin, and moxifloxacin. Like other mycoplasma, *M. genitalium* does not have a cell wall and therefore β -lactams (including penicillins and cephalosporins) are not effective treatments.

Ken B Waites. "Ureaplasma Infection Medication." *Medscape*, 2015.
<http://emedicine.medscape.com/article/231470-medication>.



Ureaplasma urealyticum microcolonies (white arrows) on agar surface after anaerobic incubation, visualized using phase contrast microscopy (800×). The black arrow indicates cellular debris. (credit: modification of work by American Society for Microbiology)

Note:

- What are the three most common causes of urethritis?
- What three members of the normal microbiota can cause urethritis?

Note:**Bacterial Infections of the Urinary Tract**

Urinary tract infections can cause inflammation of the urethra (urethritis), bladder (cystitis), and kidneys (pyelonephritis), and can sometimes spread to other body systems through the bloodstream. [\[link\]](#) captures the most important features of various types of UTIs.

Bacterial Infections of the Urinary Tract					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Cystitis	<i>Escherichia coli</i> , <i>Enterococcus faecalis</i> , <i>Streptococcus agalactiae</i> , <i>Klebsiella pneumoniae</i> , <i>Staphylococcus saprophyticus</i> , others	Dysuria, pyuria, hematuria, and bladder pain; most common in females due to the shorter urethra and abundant normal vaginal microbiota	Nontransmissible; opportunistic infections occur when fecal bacteria are introduced to urinary tract or when normal urination or immune function is impaired	Urine dipstick, urine culture for confirmation	Fluoroquinolones, nitrofurantoin, cephalosprins, trimethoprim, sulfamethoxazole
Leptospirosis	<i>Leptospira</i> spp.	Fever, headache, chills, vomiting, diarrhea, rash, muscular pain; in disseminated infections, may cause jaundice, pulmonary hemorrhaging, meningitis	From animals to humans via contact with urine or body fluids	PCR, ELISA, slide agglutination, indirect immunofluorescence	Doxycycline, amoxicillin, ampicillin, erythromycin, penicillin
Nongonococcal urethritis (NGU)	<i>Chlamydia trachomatis</i> , <i>Mycoplasma genitalium</i> , <i>Mycoplasma hominis</i> , <i>Ureaplasma urealyticum</i>	Mild or asymptomatic; may cause purulent discharge and dysuria	Transmitted sexually or from mother to neonate during birth	Urethral swabs and urine culture, PCR, NAAT	Azithromycin, doxycycline, erythromycin, fluoroquinolones
Pyelonephritis, glomerulonephritis	<i>E. coli</i> , <i>Proteus</i> spp., <i>Klebsiella</i> spp., <i>Streptococcus pyogenes</i> , others	Back pain, fever, nausea, vomiting, blood in urine; possible scarring of the kidneys and impaired kidney function; severe infections may lead to sepsis and death	Nontransmissible; infection spreads to kidneys from urinary tract or through bloodstream	Urinalysis, urine culture, radioimaging of kidneys	Penicillins, cephalosprins, fluoroquinolones, aminoglycosides, others

Key Concepts and Summary

- Bacterial **cystitis** is commonly caused by fecal bacteria such as *E. coli*.
- Pyelonephritis is a serious kidney infection that is often caused by bacteria that travel from infections elsewhere in the urinary tract and may cause systemic complications.
- **Leptospirosis** is a bacterial infection of the kidney that can be transmitted by exposure to infected animal urine, especially in contaminated water. It is more common in tropical than in temperate climates.
- **Nongonococcal urethritis (NGU)** is commonly caused by *C. trachomatis*, *M. genitalium*, *Ureaplasma urealyticum*, and *M. hominis*.
- Diagnosis and treatment for bacterial urinary tract infections varies. Urinalysis (e.g., for leukocyte esterase levels, nitrite levels, microscopic evaluation, and culture of urine) is an important component in most cases. Broad-spectrum antibiotics are typically used.

Multiple Choice

Exercise:

Problem: Which species is not associated with NGU?

- a. *Neisseria gonorrhoeae*
- b. *Mycoplasma hominis*
- c. *Chlamydia trachomatis*
- d. *Mycoplasma genitalium*

Solution:

A

Exercise:

Problem:

A strain of bacteria associated with a bladder infection shows gram-negative rods. What species is most likely to be the causative agent?

- a. *Mycoplasma hominis*
- b. *Escherichia coli*
- c. *Neisseria gonorrhoeae*
- d. *Chlamydia trachomatis*

Solution:

B

Fill in the Blank**Exercise:**

Problem: Pyelonephritis is a potentially severe infection of the ____.

Solution:

kidneys

Short Answer**Exercise:**

Problem: What is pyuria?

Critical Thinking**Exercise:**

Problem:

What are some factors that would increase an individual's risk of contracting leptospirosis?

Bacterial Infections of the Reproductive System

LEARNING OBJECTIVES

- Identify the most common bacterial pathogens that can cause infections of the reproductive system
- Compare the major characteristics of specific bacterial diseases affecting the reproductive system

In addition to infections of the urinary tract, bacteria commonly infect the reproductive tract. As with the urinary tract, parts of the reproductive system closest to the external environment are the most likely sites of infection. Often, the same microbes are capable of causing urinary tract and reproductive tract infections.

Bacterial Vaginitis and Vaginosis

Inflammation of the vagina is called vaginitis, often caused by a bacterial infection. It is also possible to have an imbalance in the normal vaginal microbiota without inflammation called **bacterial vaginosis (BV)**.

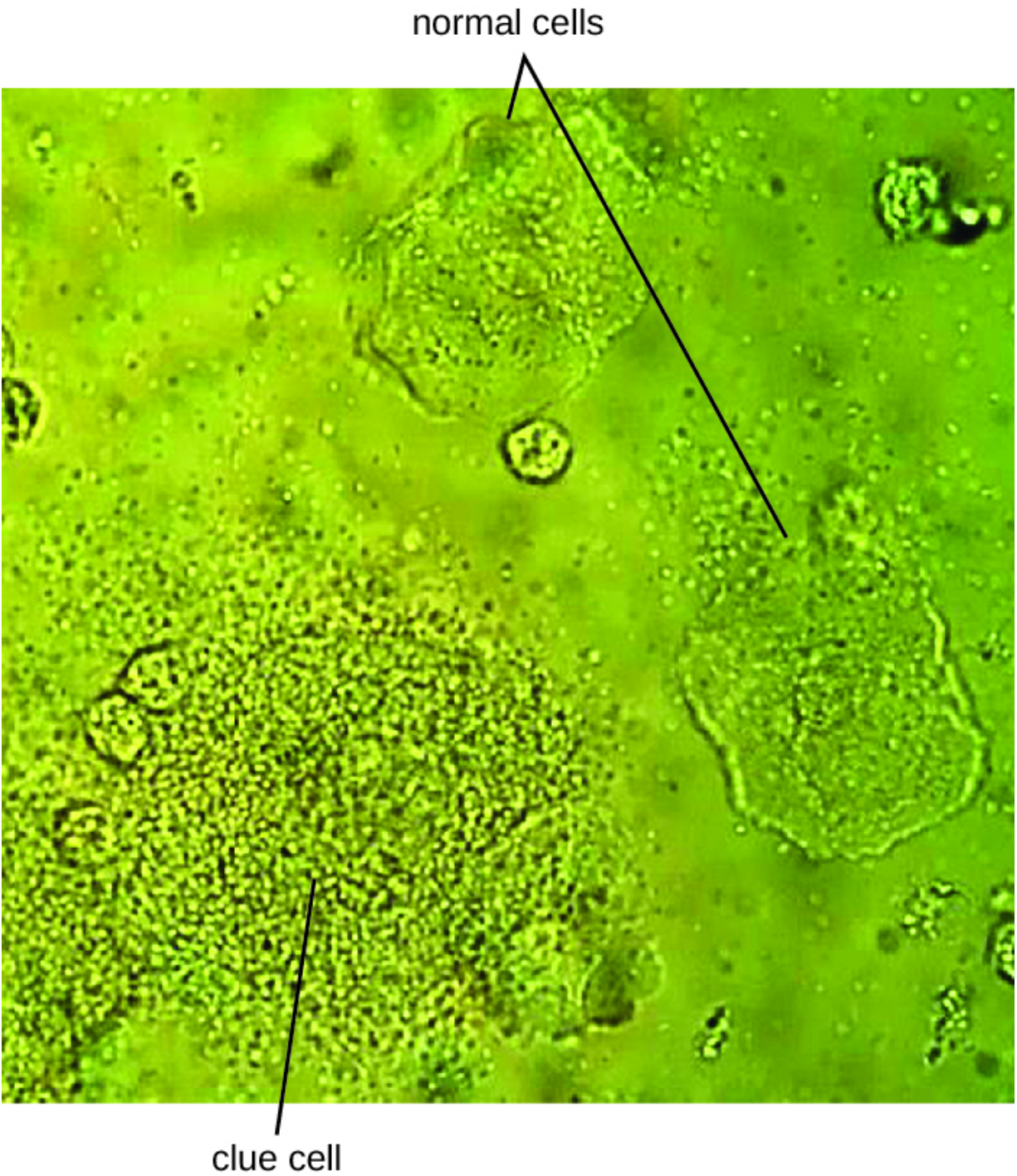
Vaginosis may be asymptomatic or may cause mild symptoms such as a thin, white-to-yellow, homogeneous vaginal discharge, burning, odor, and itching. The major causative agent is *Gardnerella vaginalis*, a gram-variable to gram-negative pleomorphic bacterium. Other causative agents include anaerobic species such as members of the genera *Bacteroides* and *Fusobacterium*. Additionally, ureaplasma and mycoplasma may be involved. The disease is usually self-limiting, although antibiotic treatment is recommended if symptoms develop.

G. vaginalis appears to be more virulent than other vaginal bacterial species potentially associated with BV. Like *Lactobacillus* spp., *G. vaginalis* is part of the normal vaginal microbiota, but when the population of *Lactobacillus* spp. decreases and the vaginal pH increases, *G. vaginalis* flourishes, causing vaginosis by attaching to vaginal epithelial cells and forming a thick protective biofilm. *G. vaginalis* also produces a cytotoxin called vaginolysin that lyses vaginal epithelial cells and red blood cells.

Since *G. vaginalis* can also be isolated from healthy women, the “gold standard” for the diagnosis of BV is direct examination of vaginal secretions and not the culture of *G. vaginalis*. Diagnosis of bacterial vaginosis from vaginal secretions can be accurately made in three ways. The first is to use a DNA probe. The second method is to assay for sialidase activity (sialidase is an enzyme produced by *G. vaginalis* and other bacteria associated with vaginosis, including *Bacteroides* spp., *Prevotella* spp., and *Mobiluncus* spp.). The third method is to assess gram-stained vaginal smears for microscopic morphology and relative numbers and types of bacteria, squamous epithelial cells, and leukocytes. By examining slides prepared from vaginal swabs, it is possible to distinguish lactobacilli (long, gram-positive rods) from other gram-negative species responsible for BV. A shift in predominance from gram-positive bacilli to gram-negative coccobacilli can indicate BV. Additionally, the slide may contain so-called clue cells, which are epithelial cells that appear to have a granular or stippled appearance due to bacterial cells attached to their surface ([\[link\]](#)). Presumptive diagnosis of bacterial vaginosis can involve an assessment of clinical symptoms and evaluation of vaginal fluids using Amsel’s diagnostic criteria which include 3 out of 4 of the following characteristics:

1. white to yellow discharge;
2. a fishy odor, most noticeable when 10% KOH is added;
3. pH greater than 4.5;
4. the presence of clue cells.

Treatment is often unnecessary because the infection often clears on its own. However, in some cases, antibiotics such as topical or oral clindamycin or metronidazole may be prescribed. Alternative treatments include oral tinidazole or clindamycin ovules (vaginal suppositories).



In this vaginal smear, the cell at the lower left is a clue cell with a unique appearance caused by the presence of bacteria on the cell. The cell on the right is a normal cell.

Note:

- Explain the difference between vaginosis and vaginitis.
- What organisms are responsible for vaginosis and what organisms typically hold it at bay?

Note:**Part 2**

There is no catch-all test for STIs, so several tests, in addition to a physical exam, are necessary to diagnose an infection. Nadia tries to relax in the exam room while she waits for the doctor to return, but she is nervous about the results.

When the doctor finally returns, she has some unexpected news: Nadia is pregnant. Surprised and excited, Nadia wants to know if the pregnancy explains her unusual symptoms. The doctor explains that the irritation that Nadia is experiencing is vaginitis, which can be caused by several types of microorganisms. One possibility is bacterial vaginosis, which develops when there is an imbalance in the bacteria in the vagina, as often occurs during pregnancy. Vaginosis can increase the risk of preterm birth and low birth weight, and a few studies have also shown that it can cause second-trimester miscarriage; however, the condition can be treated. To check for it, the doctor has asked the lab to perform a Gram stain on Nadia's sample.

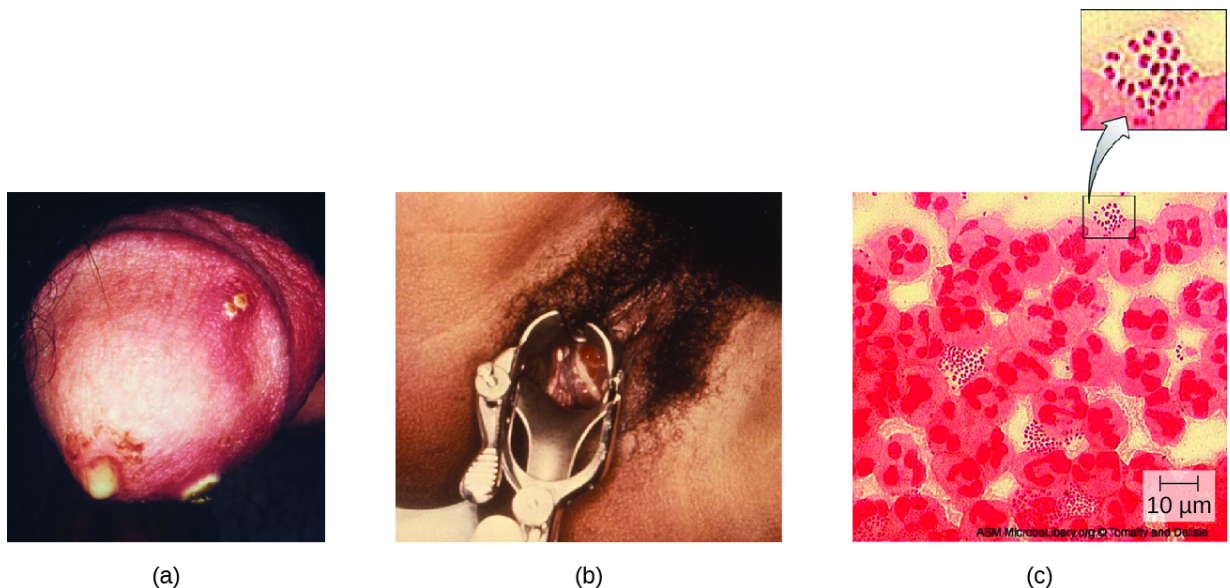
- What result would you expect from the Gram stain if Nadia has bacterial vaginosis?
- What is the relationship between pregnancy, estrogen levels, and development of bacterial vaginosis?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Gonorrhea

Also known as the clap, **gonorrhea** is a common sexually transmitted disease of the reproductive system that is especially prevalent in individuals between the ages of 15 and 24. It is caused by *Neisseria gonorrhoeae*, often called gonococcus or GC, which have fimbriae that allow the cells to attach to epithelial cells. It also has a type of lipopolysaccharide endotoxin called lipooligosaccharide as part of the outer membrane structure that enhances its pathogenicity. In addition to causing urethritis, *N. gonorrhoeae* can infect other body tissues such as the skin, meninges, pharynx, and conjunctiva.

Many infected individuals (both men and women) are asymptomatic carriers of gonorrhea. When symptoms do occur, they manifest differently in males and females. Males may develop pain and burning during urination and discharge from the penis that may be yellow, green, or white ([\[link\]](#)). Less commonly, the testicles may become swollen or tender. Over time, these symptoms can increase and spread. In some cases, chronic infection develops. The disease can also develop in the rectum, causing symptoms such as discharge, soreness, bleeding, itching, and pain (especially in association with bowel movements).



(a) Clinical photograph of gonococcal discharge from penis. The lesions on the skin could indicate co-infection with another STI. (b)

Purulent discharge originating from the cervix and accumulating in the vagina of a patient with gonorrhea. (c) A micrograph of urethral discharge shows gram-negative diplococci (paired cells) both inside and outside the leukocytes (large cells with lobed nuclei). These results could be used to diagnose gonorrhea in a male patient, but female vaginal samples may contain other *Neisseria* spp. even if the patient is not infected with *N. gonorrhoeae*. (credit a, b: modification of work by Centers for Disease Control and Prevention; credit c: modification of work by American Society for Microbiology)

Women may develop pelvic pain, discharge from the vagina, intermenstrual bleeding (i.e., bleeding not associated with normal menstruation), and pain or irritation associated with urination. As with men, the infection can become chronic. In women, however, chronic infection can cause increases in menstrual flow. Rectal infection can also occur, with the symptoms previously described for men. Infections that spread to the endometrium and fallopian tubes can cause pelvic inflammatory disease (PID), characterized by pain in the lower abdominal region, dysuria, vaginal discharge, and fever. PID can also lead to infertility through scarring and blockage of the fallopian tubes (salpingitis); it may also increase the risk of a life-threatening ectopic pregnancy, which occurs when a fertilized egg begins developing somewhere other than the uterus (e.g., in the fallopian tube or ovary).

When a gonorrhea infection disseminates throughout the body, serious complications can develop. The infection may spread through the blood (bacteremia) and affect organs throughout the body, including the heart (gonorrheal endocarditis), joints (gonorrheal arthritis), and meninges encasing the brain (meningitis).

Urethritis caused by *N. gonorrhoeae* can be difficult to treat due to antibiotic resistance (see [Micro Connections](#)). Some strains have developed resistance to the fluoroquinolones, so cephalosporins are often a first choice for treatment. Because co-infection with *C. trachomatis* is common, the CDC recommends treating with a combination regimen of ceftriaxone and

azithromycin. Treatment of sexual partners is also recommended to avoid reinfection and spread of infection to others.[\[footnote\]](#)

Centers for Disease Control and Prevention. “2015 Sexually Transmitted Diseases Treatment Guidelines: Gonococcal Infections,” 2015.

<http://www.cdc.gov/std/tg2015/gonorrhea.htm>.

Note:

- What are some of the serious consequences of a gonorrhea infection?
- What organism commonly coinfects with *N. gonorrhoeae*?

Note:

Antibiotic Resistance in *Neisseria*

Antibiotic resistance in many pathogens is steadily increasing, causing serious concern throughout the public health community. Increased resistance has been especially notable in some species, such as *Neisseria gonorrhoeae*. The CDC monitors the spread of antibiotic resistance in *N. gonorrhoeae*, which it classifies as an urgent threat, and makes recommendations for treatment. So far, *N. gonorrhoeae* has shown resistance to cefixime (a cephalosporin), ceftriaxone (another cephalosporin), azithromycin, and tetracycline. Resistance to tetracycline is the most common, and was seen in 188,600 cases of gonorrhea in 2011 (out of a total 820,000 cases). In 2011, some 246,000 cases of gonorrhea involved strains of *N. gonorrhoeae* that were resistant to at least one antibiotic.[\[footnote\]](#) These resistance genes are spread by plasmids, and a single bacterium may be resistant to multiple antibiotics. The CDC currently recommends treatment with two medications, ceftriaxone and azithromycin, to attempt to slow the spread of resistance. If resistance to cephalosporins increases, it will be extremely difficult to control the spread of *N. gonorrhoeae*.

Centers for Disease Control and Prevention. “Antibiotic Resistance Threats in the United States, 2013,” 2013.

<http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf>.

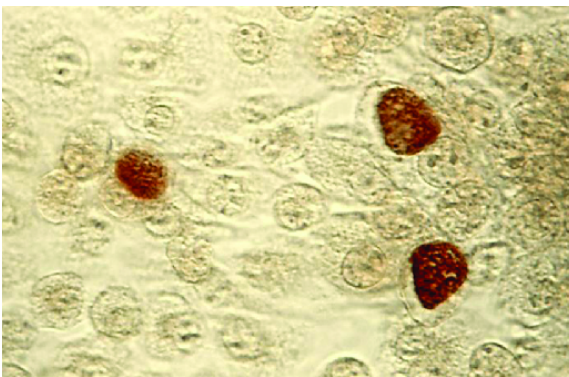
Chlamydia

Chlamydia trachomatis is the causative agent of the STI **chlamydia** ([\[link\]](#)). While many *Chlamydia* infections are asymptomatic, chlamydia is a major cause of nongonococcal urethritis (NGU) and may also cause epididymitis and orchitis in men. In women, chlamydia infections can cause urethritis, salpingitis, and PID. In addition, chlamydial infections may be associated with an increased risk of cervical cancer.

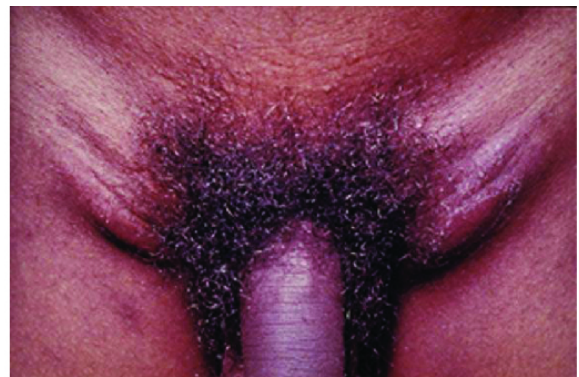
Because chlamydia is widespread, often asymptomatic, and has the potential to cause substantial complications, routine screening is recommended for sexually active women who are under age 25, at high risk (i.e., not in a monogamous relationship), or beginning prenatal care.

Certain serovars of *C. trachomatis* can cause an infection of the lymphatic system in the groin known as **lymphogranuloma venereum**. This condition is commonly found in tropical regions and can also co-occur in conjunction with human immunodeficiency virus (HIV) infection. After the microbes invade the lymphatic system, buboes (large lymph nodes, see [\[link\]](#)) form and can burst, releasing pus through the skin. The male genitals can become greatly enlarged and in women the rectum may become narrow.

Urogenital infections caused by *C. trachomatis* can be treated using azithromycin or doxycycline (the recommended regimen from the CDC). Erythromycin, levofloxacin, and ofloxacin are alternatives.



(a)



(b)

(a) *Chlamydia trachomatis* inclusion bodies within McCoy cell monolayers. Inclusion bodies are distinguished by their brown color.
(b) Lymphogranuloma venereum infection can cause swollen lymph nodes in the groin called buboes. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Herbert L. Fred and Hendrik A. van Dijk)

Note:

- Compare the signs and symptoms of chlamydia infection in men and women.

Syphilis

Syphilis is spread through direct physical (generally sexual) contact, and is caused by the gram-negative spirochete *Treponema pallidum*. *T. pallidum* has a relatively simple genome and lacks lipopolysaccharide endotoxin characteristic of gram-negative bacteria. However, it does contain lipoproteins that trigger an immune response in the host, causing tissue damage that may enhance the pathogen's ability to disseminate while evading the host immune system.

After entering the body, *T. pallidum* moves rapidly into the bloodstream and other tissues. If not treated effectively, syphilis progresses through three distinct stages: primary, secondary, and tertiary. Primary syphilis appears as a single lesion on the cervix, penis, or anus within 10 to 90 days of transmission. Such lesions contain many *T. pallidum* cells and are highly infectious. The lesion, called a **hard chancre**, is initially hard and painless, but it soon develops into an ulcerated sore ([\[link\]](#)). Localized lymph node swelling may occur as well. In some cases, these symptoms may be relatively mild, and the lesion may heal on its own within two to six weeks.

Because the lesions are painless and often occur in hidden locations (e.g., the cervix or anus), infected individuals sometimes do not notice them.

The secondary stage generally develops once the primary chancre has healed or begun to heal. Secondary syphilis is characterized by a rash that affects the skin and mucous membranes of the mouth, vagina, or anus. The rash often begins on the palms or the soles of the feet and spreads to the trunk and the limbs ([\[link\]](#)). The rash may take many forms, such as macular or papular. On mucous membranes, it may manifest as mucus patches or white, wartlike lesions called condylomata lata. The rash may be accompanied by malaise, fever, and swelling of lymph nodes. Individuals are highly contagious in the secondary stage, which lasts two to six weeks and is recurrent in about 25% of cases.

After the secondary phase, syphilis can enter a latent phase, in which there are no symptoms but microbial levels remain high. Blood tests can still detect the disease during latency. The latent phase can persist for years.

Tertiary syphilis, which may occur 10 to 20 years after infection, produces the most severe symptoms and can be fatal. Granulomatous lesions called **gummas** may develop in a variety of locations, including mucous membranes, bones, and internal organs ([\[link\]](#)). Gummas can be large and destructive, potentially causing massive tissue damage. The most deadly lesions are those of the cardiovascular system (cardiovascular syphilis) and the central nervous system (neurosyphilis). Cardiovascular syphilis can result in a fatal aortic aneurysm (rupture of the aorta) or coronary stenosis (a blockage of the coronary artery). Damage to the central nervous system can cause dementia, personality changes, seizures, general paralysis, speech impairment, loss of vision and hearing, and loss of bowel and bladder control.



(a)



(b)



(c)

(a) This ulcerated sore is a hard chancre caused by syphilis. (b) This individual has a secondary syphilis rash on the hands. (c) Tertiary syphilis produces lesions called gummas, such as this one located on the nose. (credit a, b, c: modification of work by Centers for Disease Control and Prevention)

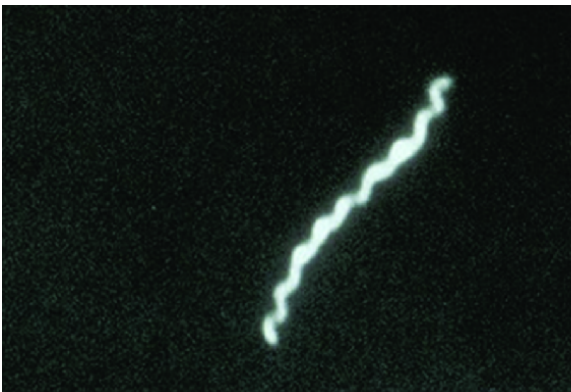
The recommended methods for diagnosing early syphilis are darkfield or brightfield (silver stain) microscopy of tissue or exudate from lesions to detect *T. pallidum* ([link](#)). If these methods are not available, two types of serologic tests (treponemal and nontreponemal) can be used for a presumptive diagnosis once the spirochete has spread in the body.

Nontreponemal serologic tests include the Venereal Disease Research Laboratory (VDRL) and rapid plasma reagin (RPR) tests. These are similar screening tests that detect nonspecific antibodies (those for lipid antigens produced during infection) rather than those produced against the spirochete. **Treponemal serologic tests** measure antibodies directed against *T. pallidum* antigens using particle agglutination (*T. pallidum* passive particle agglutination or TP-PA), immunofluorescence (the fluorescent *T. pallidum* antibody absorption or FTA-ABS), various enzyme reactions (enzyme immunoassays or EIAs) and chemiluminescence immunoassays (CIA). Confirmatory testing, rather than screening, must be done using treponemal rather than nontreponemal tests because only the former tests for antibodies to spirochete antigens. Both treponemal and nontreponemal tests should be used (as opposed to just one) since both tests have limitations than can result in false positives or false negatives.

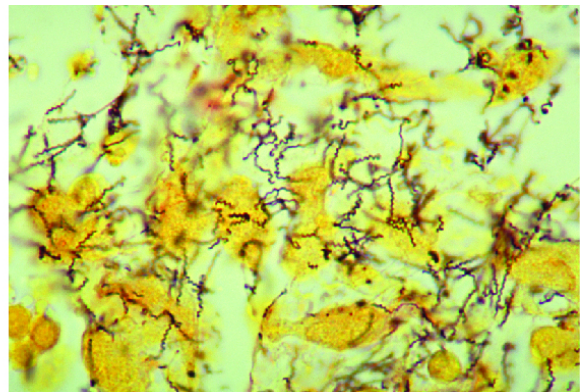
Neurosyphilis cannot be diagnosed using a single test. With or without clinical signs, it is generally necessary to assess a variety of factors,

including reactive serologic test results, cerebrospinal fluid cell count abnormalities, cerebrospinal fluid protein abnormalities, or reactive VDRL-CSF (the VDRL test of cerebrospinal fluid). The VDRL-CSF is highly specific, but not sufficiently sensitive for conclusive diagnosis.

The recommended treatment for syphilis is parenteral penicillin G (especially long-acting benzathine penicillin, although the exact choice depends on the stage of disease). Other options include tetracycline and doxycycline.



(a)



(b)

(a) Darkfield micrograph of *Treponema pallidum*. (b) Silver stain micrograph of the same species. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Congenital Syphilis

Congenital syphilis is passed by mother to fetus when untreated primary or secondary syphilis is present. In many cases, infection may lead to miscarriage or stillbirth. Children born with congenital syphilis show symptoms of secondary syphilis and may develop mucus patches that deform the nose. In infants, gummas can cause significant tissue damage to

organs and teeth. Many other complications may develop, such as osteochondritis, anemia, blindness, bone deformations, neurosyphilis, and cardiovascular lesions. Because congenital syphilis poses such a risk to the fetus, expectant mothers are screened for syphilis infection during the first trimester of pregnancy as part of the TORCH panel of prenatal tests.

Note:

- What aspect of tertiary syphilis can lead to death?
- How do treponemal serologic tests detect an infection?

Chancroid

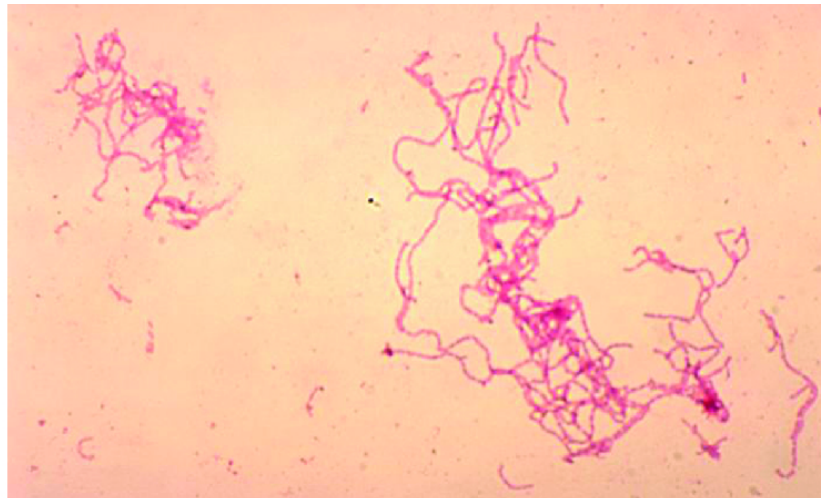
The sexually transmitted infection **chancroid** is caused by the gram-negative rod *Haemophilus ducreyi*. It is characterized by **soft chancres** ([\[link\]](#)) on the genitals or other areas associated with sexual contact, such as the mouth and anus. Unlike the hard chancres associated with syphilis, soft chancres develop into painful, open sores that may bleed or produce fluid that is highly contagious. In addition to causing chancres, the bacteria can invade the lymph nodes, potentially leading to pus discharge through the skin from lymph nodes in the groin. Like other genital lesions, soft chancres are of particular concern because they compromise the protective barriers of the skin or mucous membranes, making individuals more susceptible to HIV and other sexually transmitted diseases.

Several virulence factors have been associated with *H. ducreyi*, including lipooligosaccharides, protective outer membrane proteins, antiphagocytic proteins, secretory proteins, and collagen-specific adhesin NcaA. The collagen-specific adhesin NcaA plays an important role in initial cellular attachment and colonization. Outer membrane proteins DsrA and DltA have been shown to provide protection from serum-mediated killing by antibodies and complement.

H. ducreyi is difficult to culture; thus, diagnosis is generally based on clinical observation of genital ulcers and tests that rule out other diseases with similar ulcers, such as syphilis and genital herpes. PCR tests for *H. ducreyi* have been developed in some laboratories, but as of 2015 none had been cleared by the US Food and Drug Administration (FDA).^[footnote] Recommended treatments for chancroid include antibiotics such as azithromycin, ciprofloxacin, erythromycin and ceftriaxone. Resistance to ciprofloxacin and erythromycin has been reported.^[footnote] Centers for Disease Control and Prevention. “2015 Sexually Transmitted Diseases Treatment Guidelines: Chancroid,” 2015. <http://www.cdc.gov/std/tg2015/chancroid.htm>. Ibid.



(a)



(b)

(a) A soft chancre on the penis of a man with chancroid. (b) Chancroid is caused by the gram-negative bacterium *Haemophilus ducreyi*, seen here in a gram-stained culture of rabbit blood. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Note:

- What is the key difference between chancroid lesions and those associated with syphilis?
- Why is it difficult to definitively diagnose chancroid?

Note:**Bacterial Reproductive Tract Infections**

Many bacterial infections affecting the reproductive system are transmitted through sexual contact, but some can be transmitted by other means. In the United States, gonorrhea and chlamydia are common illnesses with incidences of about 350,000 and 1.44 million, respectively, in 2014.

Syphilis is a rarer disease with an incidence of 20,000 in 2014. Chancroid is exceedingly rare in the United States with only six cases in 2014 and a median of 10 cases per year for the years 2010–2014.[\[footnote\]](#) [\[link\]](#) summarizes bacterial infections of the reproductive tract.

Centers for Disease Control and Prevention. “2014 Sexually Transmitted Disease Surveillance,” 2015. <http://www.cdc.gov/std/stats14/default.htm>.

Bacterial Infections of the Reproductive Tract					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Bacterial vaginosis (BV)	<i>Gardnerella vaginalis</i> , <i>Bacteroides</i> spp., <i>Fusobacterium</i> spp., others	Often asymptomatic; vaginal discharge, burning, odor, or itching	Opportunistic infection caused by imbalance of normal vaginal microbiota	Vaginal smear	Clindamycin, metronidazole, tinidazole
Chancroid	<i>Haemophilus ducreyi</i>	Soft, painful chancres on genitals, mouth, or anus; swollen lymph nodes; pus discharge	Sexual contact or contact with open lesions or discharge	Observation of clinical symptoms and negative tests for syphilis and herpes	Azithromycin, ceftriaxone, erythromycin, ciprofloxacin
Chlamydia	<i>Chlamydia trachomatis</i>	Often asymptomatic; in men, urethritis, epididymitis, orchitis; in women, urethritis, vaginal discharge or bleeding, pelvic inflammatory disease, salpingitis, increased risk of cervical cancer	Sexual contact or from mother to neonate during birth	NAAT, urine sample, vaginal swab, culture	Azithromycin, doxycycline, erythromycin, ofloxacin, or levofloxacin
Gonorrhea	<i>Neisseria gonorrhoeae</i>	Urethritis, dysuria, penile or vaginal discharge, rectal pain and bleeding; in females, pelvic pain, intermenstrual bleeding, pelvic inflammatory disease, salpingitis, increased risk of infertility or ectopic pregnancy; in disseminated infections, arthritis, endocarditis, meningitis	Sexual contact	Urine sample or culture, NAAT, PCR, ELISA	Ceftriaxone, azithromycin
Syphilis	<i>Treponema pallidum</i>	Primary: hard chancre; Secondary: rash, cutaneous lesions, condylo-mata, malaise, fever, swollen lymph nodes; Tertiary: gummas, cardiovascular syphilis, neurosyphilis, possibly fatal	Sexual contact or from mother to neonate during birth	Darkfield or brightfield silver stain examination of lesion tissue or exudate, treponemal and non-treponemal serological testing, VDRL-CSF for neurosyphilis, prenatal TORCH panel	Penicillin G, tetracycline, doxycycline

Key Concepts and Summary

- **Bacterial vaginosis** is caused by an imbalance in the vaginal microbiota, with a decrease in lactobacilli and an increase in vaginal pH. *G. vaginalis* is the most common cause of bacterial vaginosis, which is associated with vaginal discharge, odor, burning, and itching.
- **Gonorrhea** is caused by *N. gonorrhoeae*, which can cause infection of the reproductive and urinary tracts and is associated with symptoms of urethritis. If left untreated, it can progress to epididymitis, salpingitis, and pelvic inflammatory disease and enter the bloodstream to infect other sites in the body.
- **Chlamydia** is the most commonly reported STI and is caused by *C. trachomatis*. Most infections are asymptomatic, and infections that are not treated can spread to involve the epididymis of men and cause salpingitis and pelvic inflammatory disease in women.
- **Syphilis** is caused by *T. pallidum* and has three stages, primary, secondary, and tertiary. Primary syphilis is associated with a painless hard chancre lesion on genitalia. Secondary syphilis is associated with skin and mucous membrane lesions. Tertiary syphilis is the most serious and life-threatening, and can involve serious nervous system damage.
- **Chancroid** is an infection of the reproductive tract caused by *H. ducreyi* that results in the development of characteristic **soft chancres**.

Multiple Choice

Exercise:

Problem:

Treponemal and non-treponemal serological testing can be used to test for

- a. vaginosis.
- b. chlamydia.
- c. syphilis.
- d. gonorrhea.

Solution:

C

Exercise:

Problem: Lymphogranuloma venereum is caused by serovars of

- a. *Neisseria gonorrhoeae*.
- b. *Chlamydia trachomatis*.
- c. *Treponema pallidum*.
- d. *Haemophilis ducreyi*.

Solution:

B

Exercise:

Problem:

The latent stage of syphilis, which may last for years, can occur between

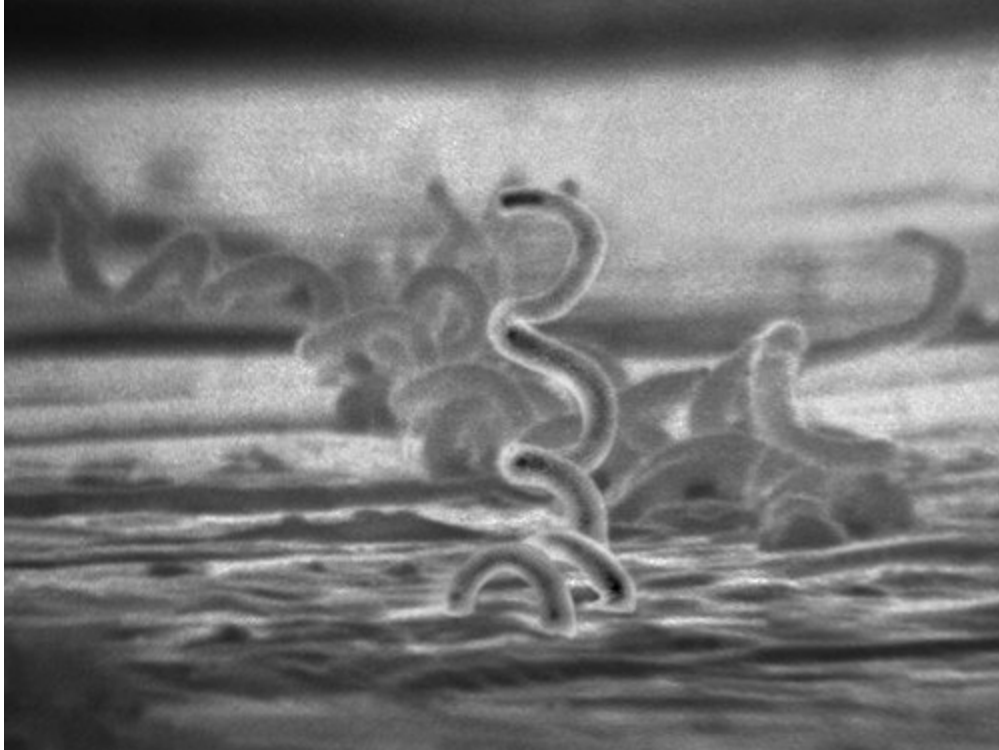
- a. the secondary and tertiary stages.
- b. the primary and secondary stages.
- c. initial infection and the primary stage.
- d. any of the three stages.

Solution:

A

Exercise:

Problem: Based on its shape, which microbe is this?



(credit: modification of work by Centers for Disease Control and Prevention)

- a. *Neisseria gonorrhoeae*
- b. *Chlamydia trachomatis*
- c. *Treponema pallidum*
- d. *Haemophilis ducreyi*

Solution:

C

Fill in the Blank

Exercise:

Problem:

Soft chancres on the genitals are characteristic of the sexually transmitted disease known as _____.

Solution:

chancroid

Short Answer**Exercise:****Problem:**

Compare gonococcal and nongonococcal urethritis with respect to their symptoms and the pathogens that cause each disease.

Critical Thinking**Exercise:****Problem:**

Chlamydia is often asymptomatic. Why might it be important for an individual to know if he or she were infected?

Exercise:**Problem:**

Why does the CDC recommend a two-drug treatment regimen to cover both *C. trachomatis* and *N. gonorrhoeae* if testing to distinguish between the two is not available? Additionally, how does the two-drug treatment regimen address antibiotic resistance?

Viral Infections of the Reproductive System

LEARNING OBJECTIVES

- Identify the most common viruses that cause infections of the reproductive system
- Compare the major characteristics of specific viral diseases affecting the reproductive system

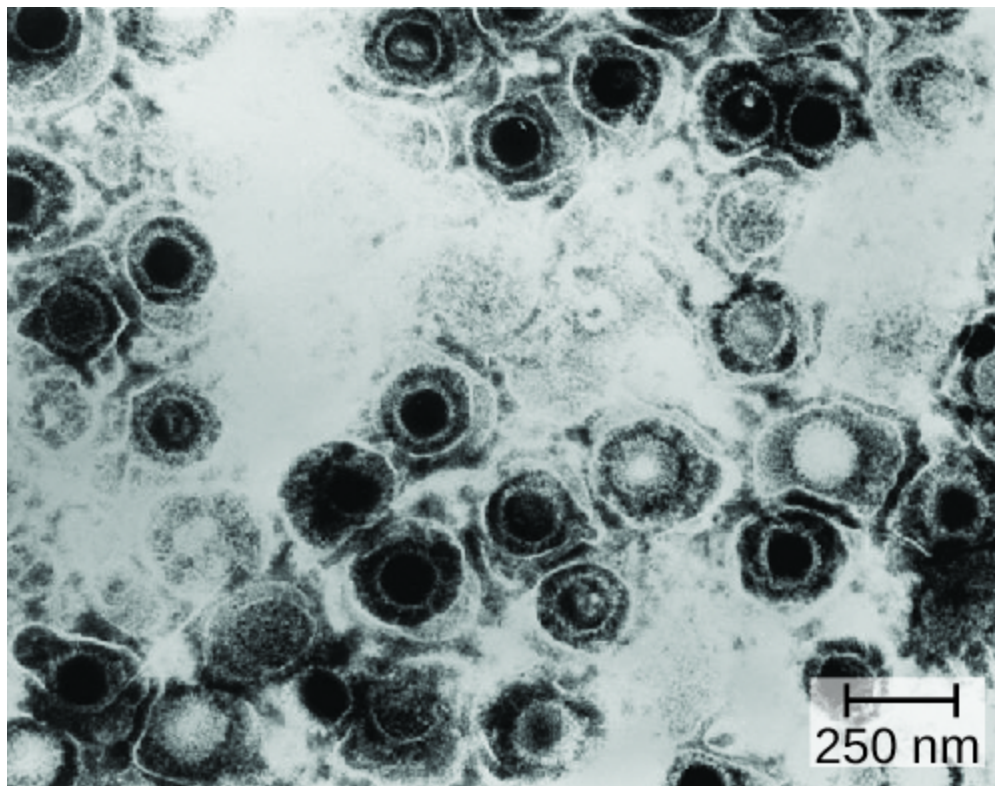
Several viruses can cause serious problems for the human reproductive system. Most of these viral infections are incurable, increasing the risk of persistent sexual transmission. In addition, such viral infections are very common in the United States. For example, human papillomavirus (HPV) is the most common STI in the country, with an estimated prevalence of 79.1 million infections in 2008; herpes simplex virus 2 (HSV-2) is the next most prevalent STI at 24.1 million infections.[\[footnote\]](#) In this section, we will examine these and other major viral infections of the reproductive system. Catherine Lindsey Satterwhite, Elizabeth Torrone, Elissa Meites, Eileen F. Dunne, Reena Mahajan, M. Cheryl Bañez Ocfemia, John Su, Fujie Xu, and Hillard Weinstock. “Sexually Transmitted Infections Among US Women and Men: Prevalence and Incidence Estimates, 2008.” *Sexually Transmitted Diseases* 40, no. 3 (2013): 187–193.

Genital Herpes

Genital herpes is a common condition caused by the herpes simplex virus ([\[link\]](#)), an enveloped, double-stranded DNA virus that is classified into two distinct types. Herpes simplex virus has several virulence factors, including infected cell protein (ICP) 34.5, which helps in replication and

inhibits the maturation of dendritic cells as a mechanism of avoiding elimination by the immune system. In addition, surface glycoproteins on the viral envelope promote the coating of herpes simplex virus with antibodies and complement factors, allowing the virus to appear as “self” and prevent immune system activation and elimination.

There are two herpes simplex virus types. While herpes simplex virus type 1 (HSV-1) is generally associated with oral lesions like cold sores or fever blisters (see [Viral Infections of the Skin and Eyes](#)), **herpes simplex virus type 2 (HSV-2)** is usually associated with genital herpes. However, both viruses can infect either location as well as other parts of the body. Oral-genital contact can spread either virus from the mouth to the genital region or vice versa.



Virions of the herpes simplex virus are shown here in this transmission electron micrograph. (credit: modification of work by Centers for Disease Control and Prevention)

Many infected individuals do not develop symptoms, and thus do not realize that they carry the virus. However, in some infected individuals, fever, chills, malaise, swollen lymph nodes, and pain precede the development of fluid-filled vesicles that may be irritating and uncomfortable. When these vesicles burst, they release infectious fluid and allow transmission of HSV. In addition, open herpes lesions can increase the risk of spreading or acquiring HIV.

In men, the herpes lesions typically develop on the penis and may be accompanied by a watery discharge. In women, the vesicles develop most commonly on the vulva, but may also develop on the vagina or cervix ([link](#)). The symptoms are typically mild, although the lesions may be irritating or accompanied by urinary discomfort. Use of condoms may not always be an effective means of preventing transmission of genital herpes since the lesions can occur on areas other than the genitals.



Genital herpes is typically characterized by lesions on the genitals (left), but lesions can also appear elsewhere on the skin or mucous membranes (right). The lesions can be large and painful or small and easily overlooked. (credit b: modification of work by Schiffer JT, Swan D, Al Sallaq R, Magaret A, Johnston C, Mark KE, Selke S, Ocbamichael N, Kuntz S, Zhu J, Robinson B, Huang ML, Jerome KR, Wald A, and Corey)

Herpes simplex viruses can cause recurrent infections because the virus can become latent and then be reactivated. This occurs more commonly with HSV-2 than with HSV-1.^[footnote] The virus moves down peripheral nerves, typically sensory neurons, to ganglia in the spine (either the trigeminal ganglion or the lumbar-sacral ganglia) and becomes latent. Reactivation can later occur, causing the formation of new vesicles. HSV-2 most effectively reactivates from the lumbar-sacral ganglia. Not everyone infected with HSV-2 experiences reactivations, which are typically associated with stressful conditions, and the frequency of reactivation varies throughout life and among individuals. Between outbreaks or when there are no obvious vesicles, the virus can still be transmitted. Centers for Disease Control and Prevention. “2015 Sexually Transmitted Disease Treatment Guidelines: Genital Herpes,” 2015. <http://www.cdc.gov/std/tg2015/herpes.htm>.

Virologic and serologic techniques are used for diagnosis. The virus may be cultured from lesions. The immunostaining methods that are used to detect virus from cultures generally require less expertise than methods based on cytopathic effect (CPE), as well as being a less expensive option. However, PCR or other DNA amplification methods may be preferred because they provide the most rapid results without waiting for culture amplification. PCR is also best for detecting systemic infections. Serologic techniques are also useful in some circumstances, such as when symptoms persist but PCR testing is negative.

While there is no cure or vaccine for HSV-2 infections, antiviral medications are available that manage the infection by keeping the virus in its dormant or latent phase, reducing signs and symptoms. If the medication is discontinued, then the condition returns to its original severity. The recommended medications, which may be taken at the start of an outbreak or daily as a method of prophylaxis, are acyclovir, famciclovir, and valacyclovir.

Neonatal Herpes

Herpes infections in newborns, referred to as **neonatal herpes**, are generally transmitted from the mother to the neonate during childbirth, when the child is exposed to pathogens in the birth canal. Infections can occur regardless of whether lesions are present in the birth canal. In most cases, the infection of the newborn is limited to skin, mucous membranes, and eyes, and outcomes are good. However, sometimes the virus becomes disseminated and spreads to the central nervous system, resulting in motor function deficits or death.

In some cases, infections can occur before birth when the virus crosses the placenta. This can cause serious complications in fetal development and may result in spontaneous abortion or severe disabilities if the fetus survives. The condition is most serious when the mother is infected with HSV for the first time during pregnancy. Thus, expectant mothers are screened for HSV infection during the first trimester of pregnancy as part of the TORCH panel of prenatal tests (see [How Pathogens Cause Disease](#)). Systemic acyclovir treatment is recommended to treat newborns with neonatal herpes.

Note:

- Why are latent herpes virus infections still of clinical concern?
- How is neonatal herpes contracted?

Human Papillomas

Warts of all types are caused by a variety of strains of **human papillomavirus (HPV)** (see [Viral Infections of the Skin and Eyes](#)). Condylomata acuminata, more commonly called **genital warts** or venereal warts ([\[link\]](#)), are an extremely prevalent STI caused by certain strains of

HPV. Condylomata are irregular, soft, pink growths that are found on external genitalia or the anus.

HPV is a small, non-enveloped virus with a circular double-stranded DNA genome. Researchers have identified over 200 different strains (called types) of HPV, with approximately 40 causing STIs. While some types of HPV cause genital warts, HPV infection is often asymptomatic and self-limiting. However, genital HPV infection often co-occurs with other STIs like syphilis or gonorrhea. Additionally, some forms of HPV (not the same ones associated with genital warts) are associated with cervical cancers. At least 14 oncogenic (cancer-causing) HPV types are known to have a causal association with cervical cancers. Examples of oncogenic HPV are types 16 and 18, which are associated with 70% of cervical cancers.[\[footnote\]](#) Oncogenic HPV types can also cause oropharyngeal cancer, anal cancer, vaginal cancer, vulvar cancer, and penile cancer. Most of these cancers are caused by HPV type 16. HPV virulence factors include proteins (E6 and E7) that are capable of inactivating tumor suppressor proteins, leading to uncontrolled cell division and the development of cancer.

Lauren Thaxton and Alan G. Waxman. “Cervical Cancer Prevention: Immunization and Screening 2015.” *Medical Clinics of North America* 99, no. 3 (2015): 469–477.

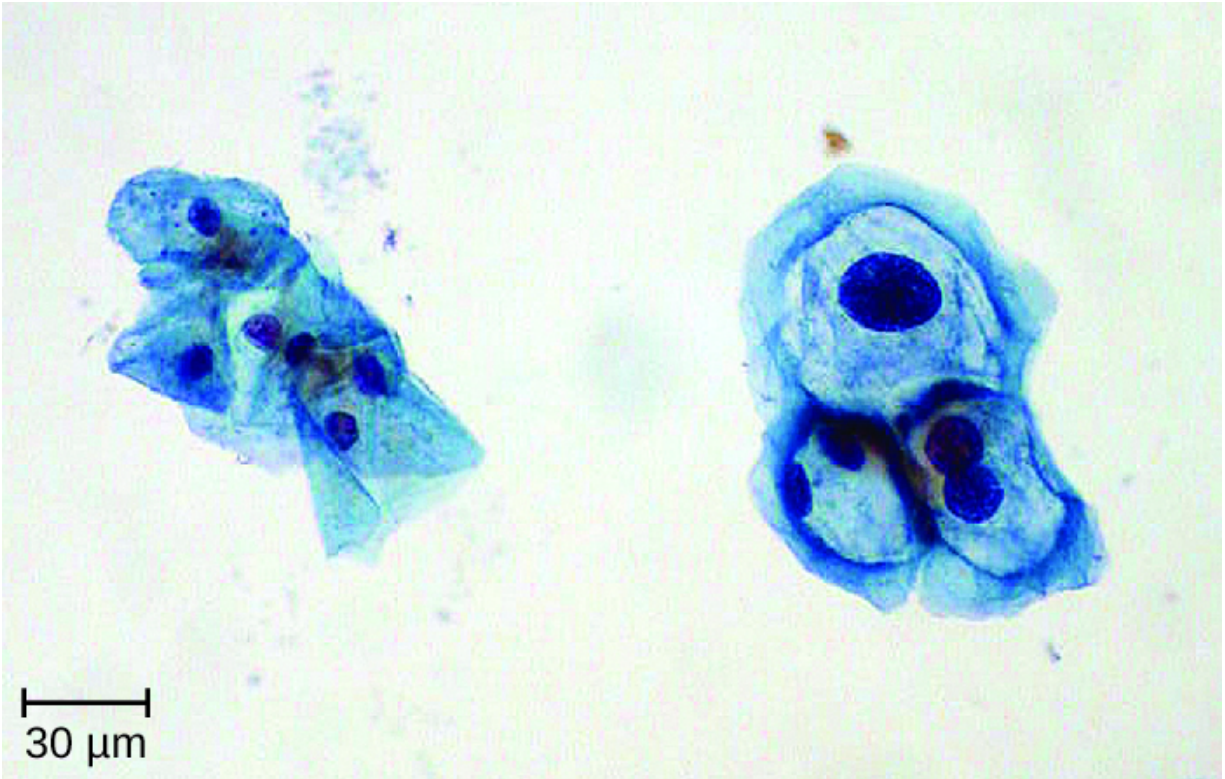
HPV cannot be cultured, so molecular tests are the primary method used to detect HPV. While routine HPV screening is not recommended for men, it is included in guidelines for women. An initial screening for HPV at age 30, conducted at the same time as a Pap test, is recommended. If the tests are negative, then further HPV testing is recommended every five years. More frequent testing may be needed in some cases. The protocols used to collect, transport, and store samples vary based on both the type of HPV testing and the purpose of the testing. This should be determined in individual cases in consultation with the laboratory that will perform the testing.

Because HPV testing is often conducted concurrently with Pap testing, the most common approach uses a single sample collection within one vial for both. This approach uses liquid-based cytology (LBC). The samples are then used for Pap smear cytology as well as HPV testing and genotyping.

HPV can be recognized in Pap smears by the presence of cells called koilocytes (called koilocytosis or koilocytotic atypia). Koilocytes have a hyperchromatic atypical nucleus that stains darkly and a high ratio of nuclear material to cytoplasm. There is a distinct clear appearance around the nucleus called a perinuclear halo ([link](#)).



Genital warts may occur around the anus (left) or genitalia (right).
(credit left, right: modification of work by Centers for Disease Control
and Prevention)



In this image, the cervical cells on the left are normal and those on the right show enlarged nuclei and hyperchromasia (darkly stained nuclei) typical of HPV-infected koilocytes. (credit: modification of work by Ed Uthman)

Most HPV infections resolve spontaneously; however, various therapies are used to treat and remove warts. Topical medications such as imiquimod (which stimulates the production of interferon), podofilox, or sinecatechins, may be effective. Warts can also be removed using cryotherapy or surgery, but these approaches are less effective for genital warts than for other types of warts. Electrocauterization and carbon dioxide laser therapy are also used for wart removal.

Regular Pap testing can detect abnormal cells that might progress to cervical cancer, followed by biopsy and appropriate treatment. Vaccines for some of the high risk HPV types are now available. Gardasil vaccine includes types 6, 11, 16 and 18 (types 6 and 11 are associated with 90% of

genital wart infections and types 16 and 18 are associated with 70% of cervical cancers). Gardasil 9 vaccinates against the previous four types and an additional five high-risk types (31, 33, 45, 52, and 58). Cervarix vaccine includes just HPV types 16 and 18. Vaccination is the most effective way to prevent infection with oncogenic HPV, but it is important to note that not all oncogenic HPV types are covered by the available vaccines. It is recommended for both boys and girls prior to sexual activity (usually between the ages of nine and fifteen).

Note:

Watch a [video](#) of how perceptions of HPV affect vaccination rates.

Note:

- What is diagnostic of an HPV infection in a Pap smear?
- What is the motivation for HPV vaccination?

Note:

Secret STIs

Few people who have an STI (or think they may have one) are eager to share that information publicly. In fact, many patients are even uncomfortable discussing the symptoms privately with their doctors.

Unfortunately, the social stigma associated with STIs makes it harder for infected individuals to seek the treatment they need and creates the false perception that STIs are rare. In reality, STIs are quite common, but it is difficult to determine exactly *how* common.

A recent study on the effects of HPV vaccination found a baseline HPV prevalence of 26.8% for women between the ages of 14 and 59. Among women aged 20–24, the prevalence was 44.8%; in other words, almost half of the women in this age bracket had a current infection.[\[footnote\]](#)

According to the CDC, HSV-2 infection was estimated to have a

prevalence of 15.5% in younger individuals (14–49 years of age) in 2007–2010, down from 20.3% in the same age group in 1988–1994. However, the CDC estimates that 87.4% of infected individuals in this age group have not been diagnosed by a physician.[\[footnote\]](#)

Eileen F. Dunne, Elizabeth R. Unger, Maya Sternberg, Geraldine McQuillan, David C. Swan, Sonya S. Patel, and Lauri E. Markowitz. “Prevalence of HPV Infection Among Females in the United States.” *Journal of the American Medical Association* 297, no. 8 (2007): 813–819.

Centers for Disease Control and Prevention. “Genital Herpes - CDC Fact Sheet,” 2015. <http://www.cdc.gov/std/herpes/stdfact-herpes-detailed.htm>.

Another complicating factor is that many STIs can be asymptomatic or have long periods of latency. For example, the CDC estimates that among women ages 14–49 in the United States, about 2.3 million (3.1%) are infected with the sexually transmitted protozoan *Trichomonas* (see [Protozoan Infections of the Urogenital System](#)); however, in a study of infected women, 85% of those diagnosed with the infection were asymptomatic.[\[footnote\]](#)

Centers for Disease Control and Prevention. “Trichomoniasis Statistics,” 2015. <http://www.cdc.gov/std/trichomonas/stats.htm>.

Even when patients are treated for symptomatic STIs, it can be difficult to obtain accurate data on the number of cases. Whereas STIs like chlamydia, gonorrhea, and syphilis are notifiable diseases—meaning each diagnosis must be reported by healthcare providers to the CDC—other STIs are not notifiable (e.g., genital herpes, genital warts, and trichomoniasis). Between the social taboos, the inconsistency of symptoms, and the lack of mandatory reporting, it can be difficult to estimate the true prevalence of STIs—but it is safe to say they are much more prevalent than most people think.

Note:

Viral Reproductive Tract Infections

[\[link\]](#) summarizes the most important features of viral diseases affecting the human reproductive tract.

Viral Infections of the Reproductive Tract					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs/Vaccines
Cervical cancer	HPV types 16, 18, and others	Development of cancer in cervix (or elsewhere)	Direct contact, including sexual	Pap smear	Gardasil vaccine, Cervarix vaccine
Genital herpes	Herpes simplex virus (HSV-1 or HSV-2)	Recurring outbreaks of skin vesicles on genitalia and elsewhere; asymptomatic in many individuals	Sexual contact or contact with open lesions	Viral culture, PCR, ELISA	Acyclovir, famciclovir, valacyclovir
Human papillomas	Human papilloma-virus (HPV) (various strains)	Genital warts or warts in other areas	Direct contact, including sexual	Pap smear	Imiquimod, podofilox, sinecatechins
Neonatal herpes	Herpes simplex virus (HSV-1 or HSV-2)	Vesicles on the skin, mucous membranes, eyes; in disseminated infections, motor impairment and possible death of fetus or newborn	Exposure to pathogens in the birth canal; transplacental infection in some cases	Viral culture or PCR	Acyclovir

Key Concepts and Summary

- **Genital herpes** is usually caused by **HSV-2** (although HSV-1 can also be responsible) and may cause the development of infectious, potentially recurrent vesicles
- **Neonatal herpes** can occur in babies born to infected mothers and can cause symptoms that range from relatively mild (more common) to severe.
- **Human papillomaviruses** are the most common sexually transmitted viruses and include strains that cause **genital warts** as well as strains that cause **cervical cancer**.

Multiple Choice

Exercise:

Problem: Genital herpes is most commonly caused by

- a. herpes simplex virus 1.
- b. varicella-zoster virus.
- c. herpes simplex virus 2.
- d. cytomegalovirus.

Solution:

C

Exercise:

Problem: Koilocytes are characteristic of

- A. cells infected with human papillomavirus
- B. cells infected with herpes simplex virus 2
- C. cells infected with all forms of herpesviruses
- D. cervical cancer cells

Solution:

A

Fill in the Blank**Exercise:**

Problem: Condylomata are _____.

Solution:

warts

Short Answer

Exercise:

Problem:

Is it true that human papillomaviruses can always be detected by the presence of genital warts?

Exercise:

Problem: How is neonatal herpes transmitted?

Critical Thinking

Exercise:

Problem:

Recently, studies have shown a reduction in the prevalence of some strains of HPV in younger women. What might be the reason for this?

Fungal Infections of the Reproductive System

LEARNING OBJECTIVES

- Summarize the important characteristics of vaginal candidiasis

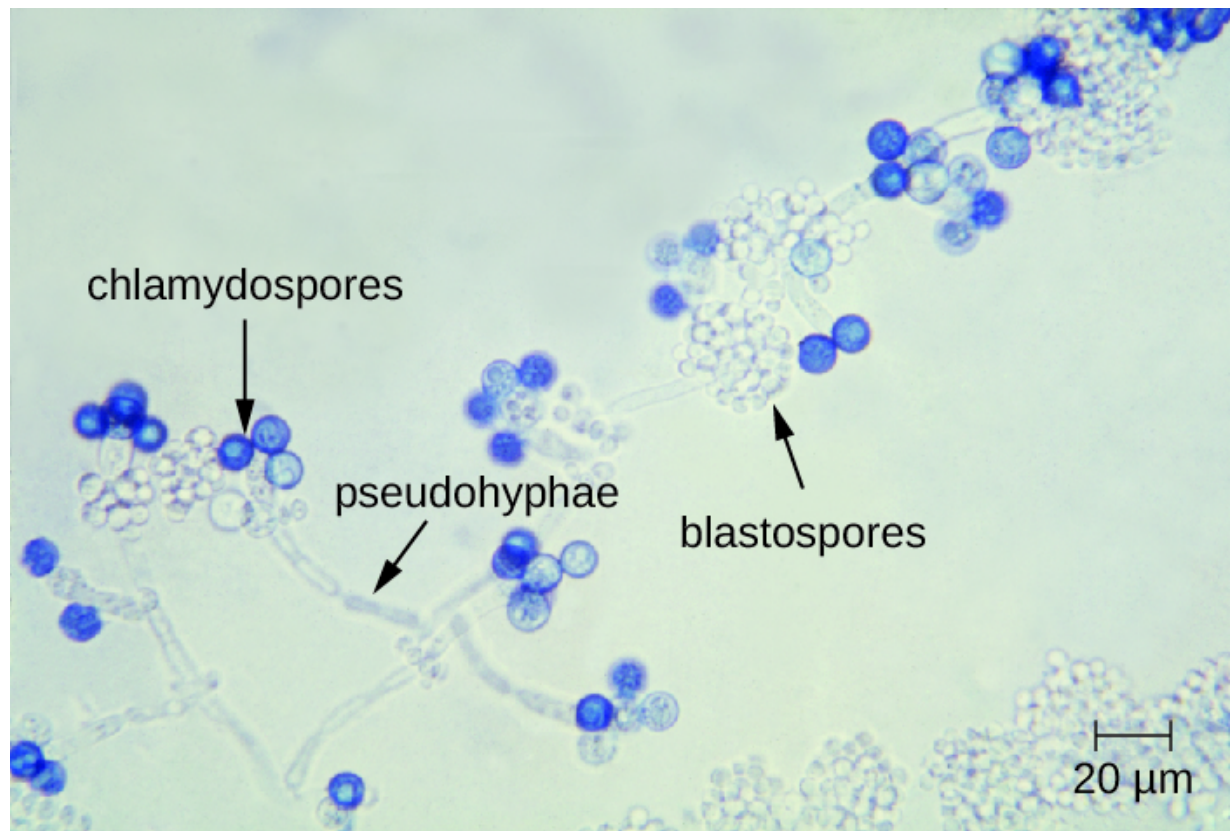
Only one major fungal pathogen affects the urogenital system. *Candida* is a genus of fungi capable of existing in a yeast form or as a multicellular fungus. *Candida* spp. are commonly found in the normal, healthy microbiota of the skin, gastrointestinal tract, respiratory system, and female urogenital tract ([link](#)). They can be pathogenic due to their ability to adhere to and invade host cells, form biofilms, secrete hydrolases (e.g., proteases, phospholipases, and lipases) that assist in their spread through tissues, and change their phenotypes to protect themselves from the immune system. However, they typically only cause disease in the female reproductive tract under conditions that compromise the host's defenses. While there are at least 20 *Candida* species of clinical importance, *C. albicans* is the species most commonly responsible for fungal vaginitis.

As discussed earlier, lactobacilli in the vagina inhibit the growth of other organisms, including bacteria and *Candida*, but disruptions can allow *Candida* to increase in numbers. Typical disruptions include antibiotic therapy, illness (especially diabetes), pregnancy, and the presence of transient microbes. Immunosuppression can also play a role, and the severe immunosuppression associated with HIV infection often allows *Candida* to thrive. This can cause genital or vaginal **candidiasis**, a condition characterized by vaginitis and commonly known as a yeast infection. When a yeast infection develops, inflammation occurs along with symptoms of pruritus (itching), a thick white or yellow discharge, and odor.

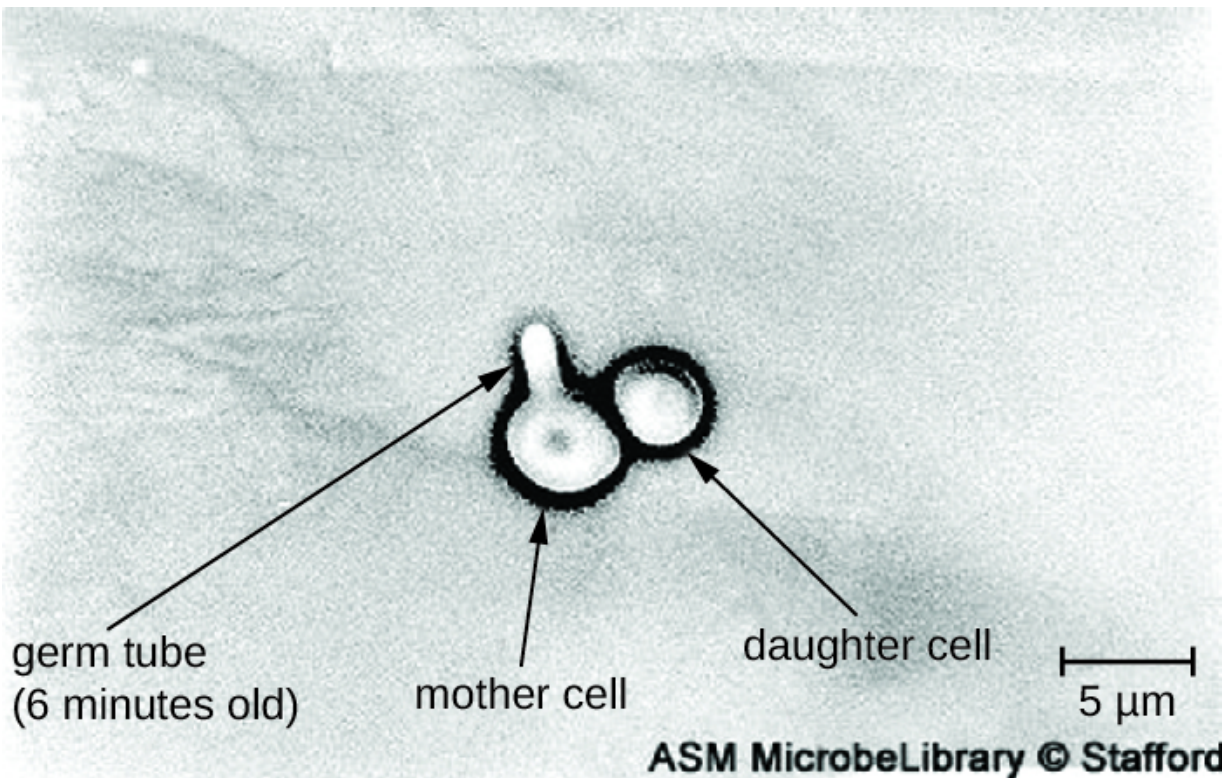
Other forms of candidiasis include cutaneous candidiasis (see [Mycoses of the Skin](#)) and oral thrush (see [Microbial Diseases of the Mouth and Oral Cavity](#)). Although *Candida* spp. are found in the normal microbiota, *Candida* spp. may also be transmitted between individuals. Sexual contact is a common mode of transmission, although candidiasis is not considered an STI.

Diagnosis of vaginal candidiasis can be made using microscopic evaluation of vaginal secretions to determine whether there is an excess of *Candida*. Culturing approaches are less useful because *Candida* is part of the normal microbiota and will regularly appear. It is also easy to contaminate samples with *Candida* because it is so common, so care must be taken to handle clinical material appropriately. Samples can be refrigerated if there is a delay in handling. *Candida* is a dimorphic fungus, so it does not only exist in a yeast form; cultivation can be used to identify chlamydospores and pseudohyphae, which develop from germ tubes ([link](#)). The presence of the germ tube can be used in a diagnostic test in which cultured yeast cells are combined with rabbit serum and observed after a few hours for the presence of germ tubes. Molecular tests are also available if needed. The Affirm VPII Microbial Identification Test, for instance, tests simultaneously for the vaginal microbes *C. albicans*, *G. vaginalis* (see [Bacterial Infections of the Urinary System](#)), and *Trichomonas vaginalis* (see [Protozoan Infections of the Urogenital System](#)).

Topical antifungal medications for vaginal candidiasis include butoconazole, miconazole, clotrimazole, tioconazole, and nystatin. Oral treatment with fluconazole can be used. There are often no clear precipitating factors for infection, so prevention is difficult.



Candida blastospores (asexual spores that result from budding) and chlamydo spores (resting spores produced through asexual reproduction) are visible in this micrograph. (credit: modification of work by Centers for Disease Control and Prevention)



Candida can produce germ tubes, like the one in this micrograph, that develop into hyphae. (credit: modification of work by American Society for Microbiology)

Note:

- What factors can lead to candidiasis?
- How is candidiasis typically diagnosed?

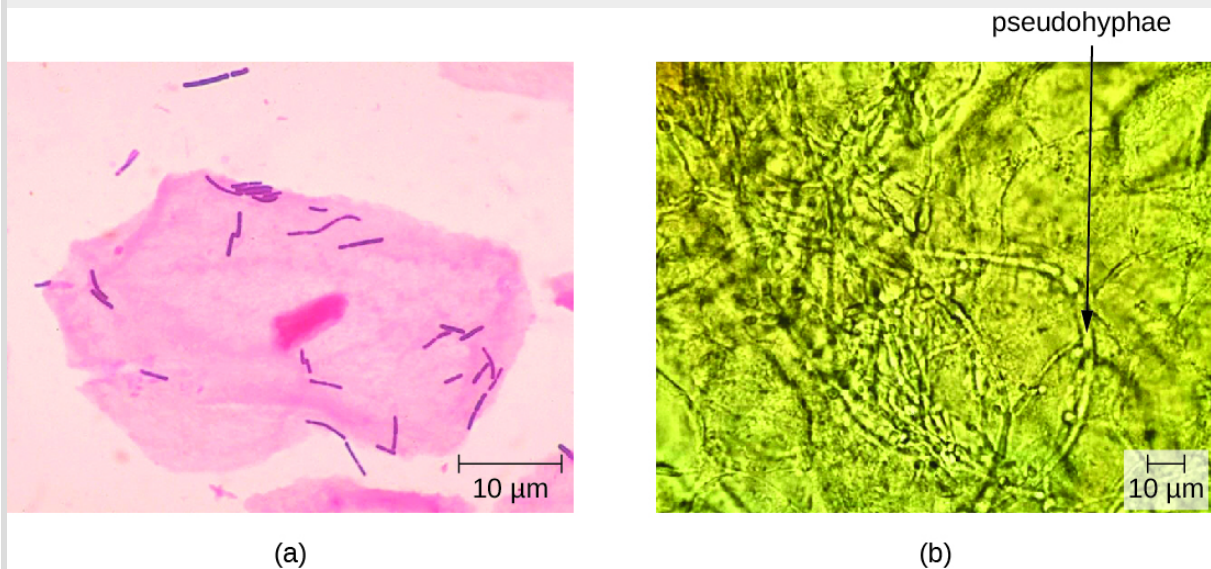
Note:

Part 3

The Gram stain of Nadia's vaginal smear showed that the concentration of lactobacilli relative to other species in Nadia's vaginal sample was

abnormally low. However, there were no clue cells visible, which suggests that the infection is not bacterial vaginosis. But a wet-mount slide showed an overgrowth of yeast cells, suggesting that the problem is candidiasis, or a yeast infection ([\[link\]](#)). This, Nadia's doctor assures her, is good news. Candidiasis is common during pregnancy and easily treatable.

- Knowing that the problem is candidiasis, what treatments might the doctor suggest?



(a) Lactobacilli are visible as gram-positive rods on and around this squamous epithelial cell. (b) This wet mount prepared with KOH shows *Candida albicans* pseudohyphae and squamous epithelial cells in a vaginal sample from a patient with candidiasis. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Mikael Häggström)

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- *Candida* spp. are typically present in the normal microbiota in the body, including the skin, respiratory tract, gastrointestinal tract, and female urogenital system.
- Disruptions in the normal vaginal microbiota can lead to an overgrowth of *Candida*, causing vaginal **candidiasis**.
- Vaginal candidiasis can be treated with topical or oral fungicides. Prevention is difficult.

Multiple Choice

Exercise:

Problem:

Which oral medication is recommended as an initial topical treatment for genital yeast infections?

- a. penicillin
- b. acyclovir
- c. fluconazole
- d. miconazole

Solution:

D

Fill in the Blank

Exercise:

Problem:

The most common *Candida* species associated with yeast infections is _____.

Solution:

C. albicans

Protozoan Infections of the Urogenital System

LEARNING OBJECTIVES

- Identify the most common protozoan pathogen that causes infections of the reproductive system
- Summarize the important characteristics of trichomoniasis

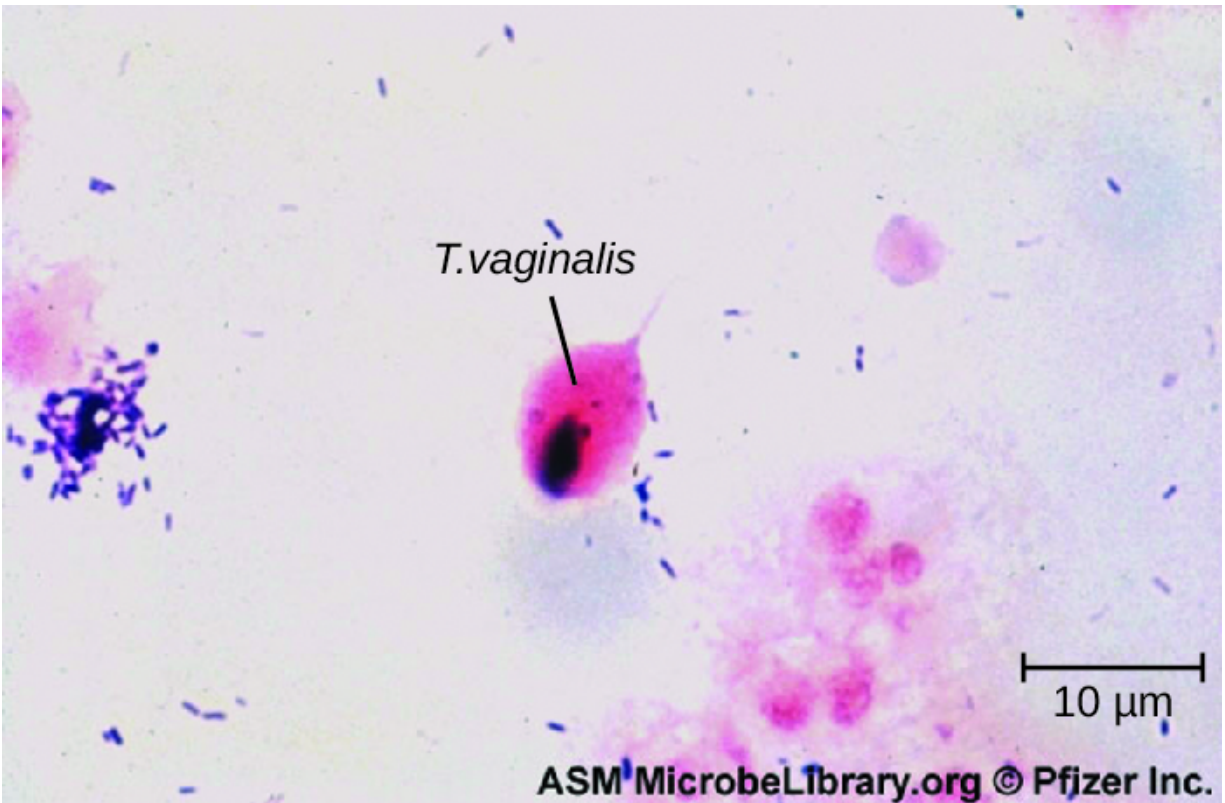
Only one major protozoan species causes infections in the urogenital system. **Trichomoniasis**, or “trich,” is the most common nonviral STI and is caused by a flagellated protozoan *Trichomonas vaginalis*. *T. vaginalis* has an undulating membrane and, generally, an amoeboid shape when attached to cells in the vagina. In culture, it has an oval shape.

T. vaginalis is commonly found in the normal microbiota of the vagina. As with other vaginal pathogens, it can cause vaginitis when there is disruption to the normal microbiota. It is found only as a trophozoite and does not form cysts. *T. vaginalis* can adhere to cells using adhesins such as lipoglycans; it also has other cell-surface virulence factors, including tetraspanins that are involved in cell adhesion, motility, and tissue invasion. In addition, *T. vaginalis* is capable of phagocytosing other microbes of the normal microbiota, contributing to the development of an imbalance that is favorable to infection.

Both men and women can develop trichomoniasis. Men are generally asymptomatic, and although women are more likely to develop symptoms, they are often asymptomatic as well. When symptoms do occur, they are characteristic of urethritis. Men experience itching, irritation, discharge from the penis, and burning after urination or ejaculation. Women

experience dysuria; itching, burning, redness, and soreness of the genitalia; and vaginal discharge. The infection may also spread to the cervix. Infection increases the risk of transmitting or acquiring HIV and is associated with pregnancy complications such as preterm birth.

Microscopic evaluation of wet mounts is an inexpensive and convenient method of diagnosis, but the sensitivity of this method is low ([\[link\]](#)). Nucleic acid amplification testing (NAAT) is preferred due to its high sensitivity. Using wet mounts and then NAAT for those who initially test negative is one option to improve sensitivity. Samples may be obtained for NAAT using urine, vaginal, or endocervical specimens for women and with urine and urethral swabs for men. It is also possible to use other methods such as the OSOM *Trichomonas* Rapid Test (an immunochromatographic test that detects antigen) and a DNA probe test for multiple species associated with vaginitis (the Affirm VPII Microbial Identification Test discussed in section 23.5).[\[footnote\]](#) *T. vaginalis* is sometimes detected on a Pap test, but this is not considered diagnostic due to high rates of false positives and negatives. The recommended treatment for trichomoniasis is oral metronidazole or tinidazole. Sexual partners should be treated as well. Association of Public Health Laboratories. “Advances in Laboratory Detection of *Trichomonas vaginalis*,” 2013. http://www.aphl.org/AboutAPHL/publications/Documents/ID_2013August_Advances-in-Laboratory-Detection-of-Trichomonas-vaginalis.pdf.



Trichomonas vaginalis is visible in this Gram stained specimen.
(credit: modification of work by American Society for Microbiology)

Note:

- What are the symptoms of trichomoniasis?

Note:

STIs and Privacy

For many STIs, it is common to contact and treat sexual partners of the patient. This is especially important when a new illness has appeared, as when HIV became more prevalent in the 1980s. But to contact sexual

partners, it is necessary to obtain their personal information from the patient. This raises difficult questions. In some cases, providing the information may be embarrassing or difficult for the patient, even though withholding such information could put their sexual partner(s) at risk. Legal considerations further complicate such situations. The Health Insurance Portability and Accountability Act (HIPPA), passed into law in 1996, sets the standards for the protection of patient information. It requires businesses that use health information, such as insurance companies and healthcare providers, to maintain strict confidentiality of patient records. Contacting a patient's sexual partners may therefore violate the patient's privacy rights if the patient's diagnosis is revealed as a result. From an ethical standpoint, which is more important: the patient's privacy rights or the sexual partner's right to know that they may be at risk of a sexually transmitted disease? Does the answer depend on the severity of the disease or are the rules universal? Suppose the physician knows the identity of the sexual partner but the patient does not want that individual to be contacted. Would it be a violation of HIPPA rules to contact the individual without the patient's consent?

Questions related to patient privacy become even more complicated when dealing with patients who are minors. Adolescents may be reluctant to discuss their sexual behavior or health with a health professional, especially if they believe that healthcare professionals will tell their parents. This leaves many teens at risk of having an untreated infection or of lacking the information to protect themselves and their partners. On the other hand, parents may feel that they have a right to know what is going on with their child. How should physicians handle this? Should parents always be told even if the adolescent wants confidentiality? Does this affect how the physician should handle notifying a sexual partner?

Note:**Resolution**

Vaginal candidiasis is generally treated using topical antifungal medications such as butoconazole, miconazole, clotrimazole, ticonazole, nystatin, or oral fluconazole. However, it is important to be careful in selecting a treatment for use during pregnancy. Nadia's doctor

recommended treatment with topical clotrimazole. This drug is classified as a category B drug by the FDA for use in pregnancy, and there appears to be no evidence of harm, at least in the second or third trimesters of pregnancy. Based on Nadia's particular situation, her doctor thought that it was suitable for very short-term use even though she was still in the first trimester. After a seven-day course of treatment, Nadia's yeast infection cleared. She continued with a normal pregnancy and delivered a healthy baby eight months later.

Higher levels of hormones during pregnancy can shift the typical microbiota composition and balance in the vagina, leading to high rates of infections such as candidiasis or vaginosis. Topical treatment has an 80–90% success rate, with only a small number of cases resulting in recurrent or persistent infections. Longer term or intermittent treatment is usually effective in these cases.

Go back to the [previous](#) Clinical Focus box.

Note:**Fungal and Protozoan Reproductive Tract Infections**

[\[link\]](#) summarizes the most important features of candidiasis and trichomoniasis.

Fungal and Protozoan Infections of the Reproductive Tract					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Trichomoniasis	<i>Trichomonas vaginalis</i>	Urethritis, vaginal or penile discharge; redness or soreness of female genitalia	Sexual contact	Wet mounts, NAAT of urine or vaginal samples; OSOM Trichomonas Rapid Test, Affirm VPII Microbial Identification Test	Metronidazole, tinidazole
Vaginal candidiasis (yeast infection)	<i>Candida</i> spp., especially <i>C. albicans</i>	Dysuria; vaginal burning, itching, discharge	Transmissible by sexual contact, but typically only causes opportunistic infections after immunosuppression or disruption of vaginal microbiota	Culture, Affirm VPII Microbial Identification Test	Fluconazole, miconazole, clotrimazole, tioconazole, nystatin

Note:

Take an [online quiz](#) for a review of sexually transmitted infections.

Key Concepts and Summary

- **Trichomoniasis** is a common STI caused by *Trichomonas vaginalis*.
- *T. vaginalis* is common at low levels in the normal microbiota.
- Trichomoniasis is often asymptomatic. When symptoms develop, trichomoniasis causes urinary discomfort, irritation, itching, burning, discharge from the penis (in men), and vaginal discharge (in women).
- Trichomoniasis is treated with the antflagellate drugs tinidazole and metronidazole.

Multiple Choice

Exercise:

Problem:

What is the only common infection of the reproductive tract caused by a protozoan?

- a. gonorrhea
- b. chlamydia
- c. trichomoniasis
- d. candidiasis

Solution:

C

Exercise:

Problem:

Which test is preferred for detecting *T. vaginalis* because of its high sensitivity?

- a. NAAT
- b. wet mounts
- c. Pap tests
- d. all of the above are equally good

Solution:

A

Fill in the Blank

Exercise:

Problem: Trichomoniasis is caused by ____.

Solution:

Trichomonas vaginalis

Short Answer

Exercise:

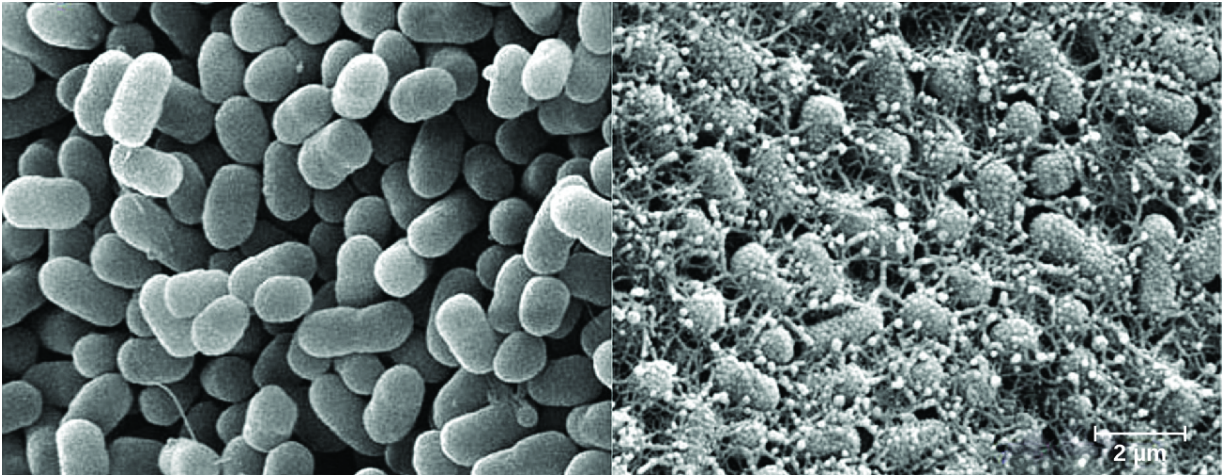
Problem:

Name three organisms (a bacterium, a fungus, and a protozoan) that are associated with vaginitis.

Introduction

class="introduction"

E. coli
O157:H7
causes
serious
foodborne
illness.
Curli fibers
(adhesive
surface
fibers that
are part of
the
extracellular
matrix) help
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Pictured are
two groups
of cells,
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and curli
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cells (right).
(credit left,
right:
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by USDA)



Gastrointestinal (GI) diseases are so common that, unfortunately, most people have had first-hand experience with the unpleasant symptoms, such as diarrhea, vomiting, and abdominal discomfort. The causes of gastrointestinal illness can vary widely, but such diseases can be grouped into two categories: those caused by infection (the growth of a pathogen in the GI tract) or intoxication (the presence of a microbial toxin in the GI tract).

Foodborne pathogens like *Escherichia coli* O157:H7 are among the most common sources of gastrointestinal disease. Contaminated food and water have always posed a health risk for humans, but in today's global economy, outbreaks can occur on a much larger scale. *E. coli* O157:H7 is a potentially deadly strain of *E. coli* with a history of contaminating meat and produce that are not properly processed. The source of an *E. coli* O157:H7 outbreak can be difficult to trace, especially if the contaminated food is processed in a foreign country. Once the source is identified, authorities may issue recalls of the contaminated food products, but by then there are typically numerous cases of food poisoning, some of them fatal.

Anatomy and Normal Microbiota of the Digestive System

LEARNING OBJECTIVES

- Describe the major anatomical features of the human digestive system
- Describe the normal microbiota of various regions in the human digestive system
- Explain how microorganisms overcome the defenses of the digestive tract to cause infection or intoxication
- Describe general signs and symptoms associated with infections of the digestive system

Note:

Part 1

After a morning of playing outside, four-year-old Carli ran inside for lunch. After taking a bite of her fried egg, she pushed it away and whined, “It’s too slimy, Mommy. I don’t want any more.” But her mother, in no mood for games, curtly replied that if she wanted to go back outside she had better finish her lunch. Reluctantly, Carli complied, trying hard not to gag as she choked down the runny egg.

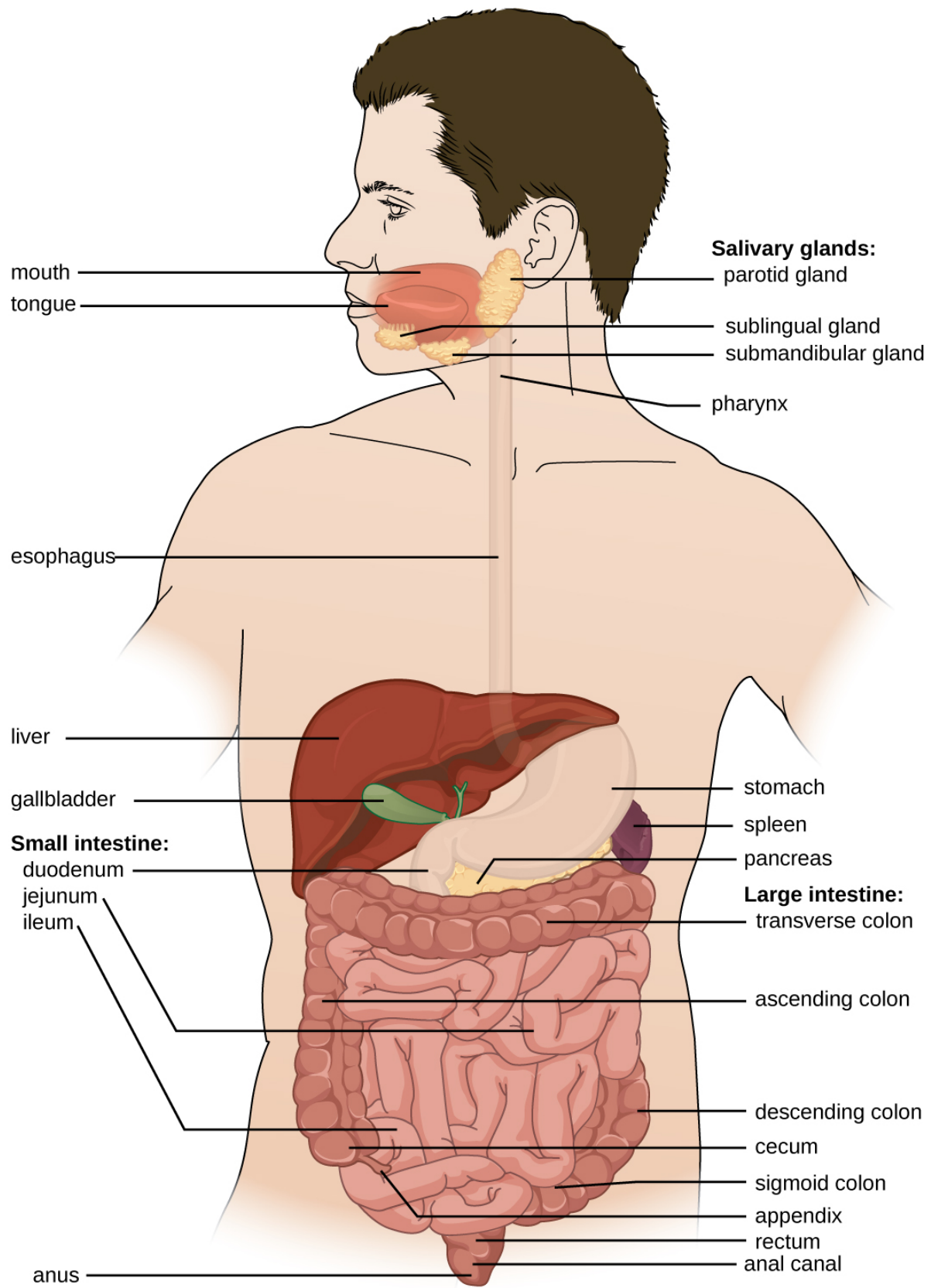
That night, Carli woke up feeling nauseated. She cried for her parents and then began to vomit. Her parents tried to comfort her, but she continued to vomit all night and began to have diarrhea and run a fever. By the morning, her parents were very worried. They rushed her to the emergency room.

- What could have caused Carli’s signs and symptoms?

Jump to the [next](#) Clinical Focus box.

The human digestive system, or the gastrointestinal (GI) tract, begins with the mouth and ends with the anus. The parts of the mouth include the teeth, the gums, the tongue, the oral vestibule (the space between the gums, lips, and teeth), and the oral cavity proper (the space behind the teeth and gums). Other parts of the GI tract are the pharynx, esophagus, stomach, small intestine, large intestine, rectum, and anus ([\[link\]](#)). Accessory digestive organs include the salivary glands, liver, gallbladder, spleen, and pancreas.

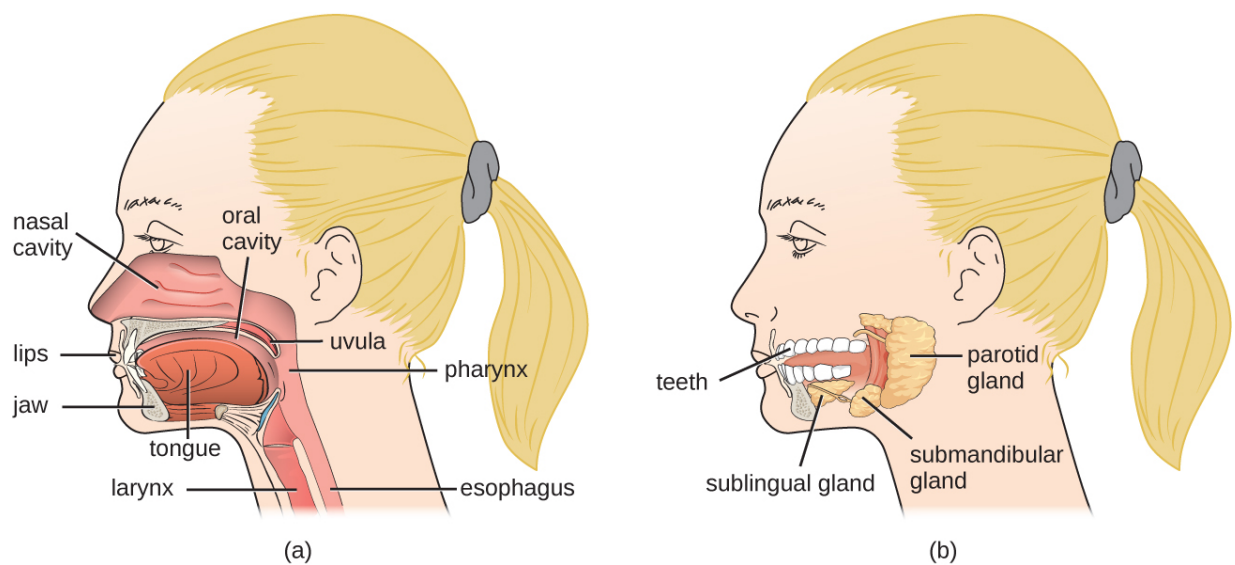
The digestive system contains normal microbiota, including archaea, bacteria, fungi, protists, and even viruses. Because this microbiota is important for normal functioning of the digestive system, alterations to the microbiota by antibiotics or diet can be harmful. Additionally, the introduction of pathogens to the GI tract can cause infections and diseases. In this section, we will review the microbiota found in a healthy digestive tract and the general signs and symptoms associated with oral and GI infections.



The digestive system, or the gastrointestinal tract, includes all of the organs associated with the digestion of food.

Anatomy and Normal Microbiota of the Oral Cavity

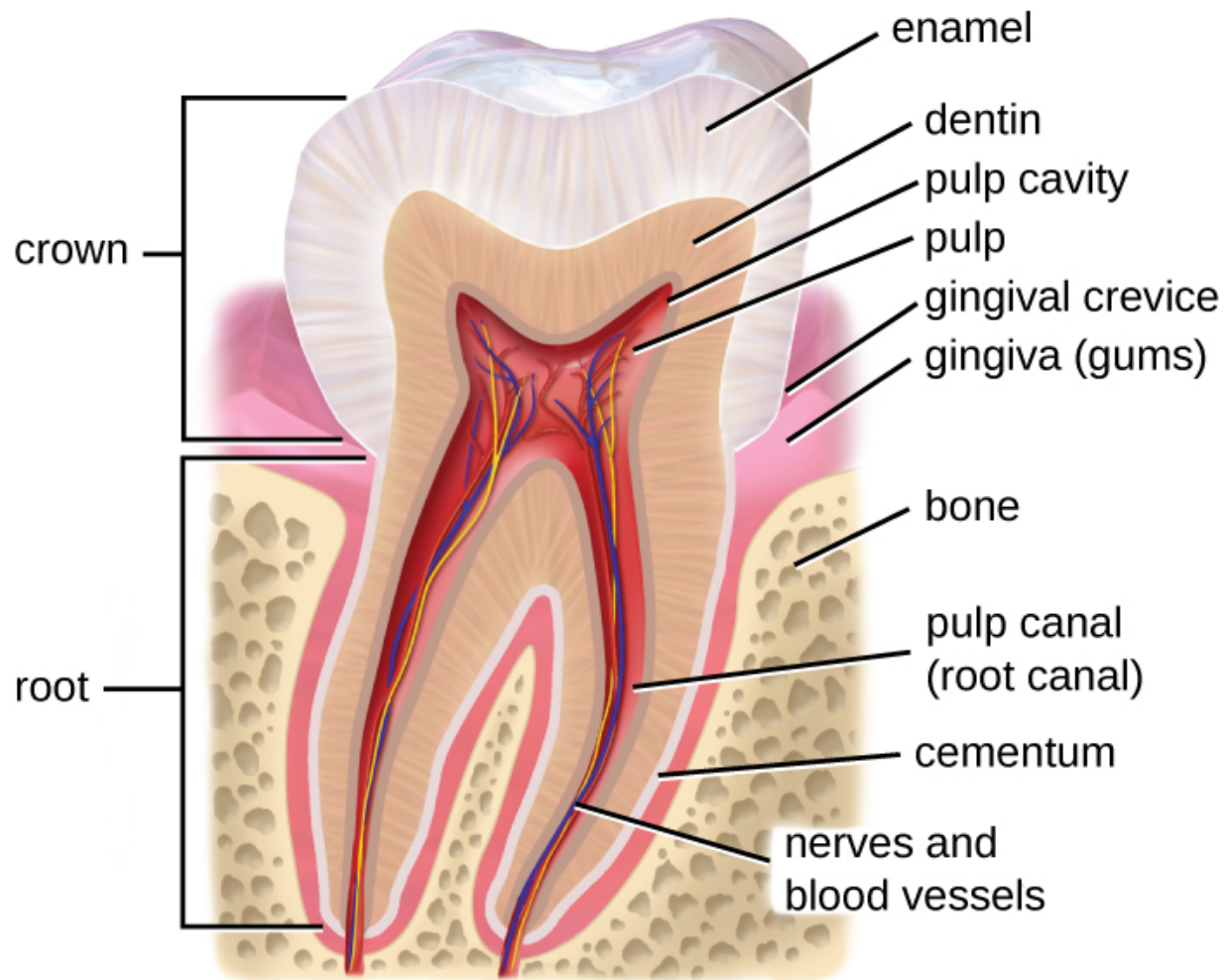
Food enters the digestive tract through the mouth, where mechanical digestion (by chewing) and chemical digestion (by enzymes in saliva) begin. Within the mouth are the tongue, teeth, and salivary glands, including the parotid, sublingual, and submandibular glands ([link](#)). The salivary glands produce saliva, which lubricates food and contains digestive enzymes.



(a) When food enters the mouth, digestion begins. (b) Salivary glands are accessory digestive organs. (credit: modification of work by National Cancer Institute)

The structure of a tooth ([link](#)) begins with the visible outer surface, called the crown, which has to be extremely hard to withstand the force of biting

and chewing. The crown is covered with enamel, which is the hardest material in the body. Underneath the crown, a layer of relatively hard dentin extends into the root of the tooth around the innermost pulp cavity, which includes the pulp chamber at the top of the tooth and pulp canal, or root canal, located in the root. The pulp that fills the pulp cavity is rich in blood vessels, lymphatic vessels, connective tissue, and nerves. The root of the tooth and some of the crown are covered with cementum, which works with the periodontal ligament to anchor the tooth in place in the jaw bone. The soft tissues surrounding the teeth and bones are called gums, or gingiva. The gingival space or gingival crevice is located between the gums and teeth.



The tooth has a visible crown with an outer layer of enamel, a layer of dentin, and an inner pulp. The root, hidden by the gums, contains the pulp canal (root canal). (credit: modification of work by Bruce Blaus)

Microbes such as bacteria and archaea are abundant in the mouth and coat all of the surfaces of the oral cavity. However, different structures, such as the teeth or cheeks, host unique communities of both aerobic and anaerobic microbes. Some factors appear to work against making the mouth hospitable to certain microbes. For example, chewing allows microbes to mix better with saliva so they can be swallowed or spit out more easily. Saliva also contains enzymes, including lysozyme, which can damage microbial cells. Recall that lysozyme is part of the first line of defense in the innate immune system and cleaves the β -(1,4) glycosidic linkages between N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) in bacterial peptidoglycan (see [Chemical Defenses](#)). Additionally, fluids containing immunoglobulins and phagocytic cells are produced in the gingival spaces. Despite all of these chemical and mechanical activities, the mouth supports a large microbial community.

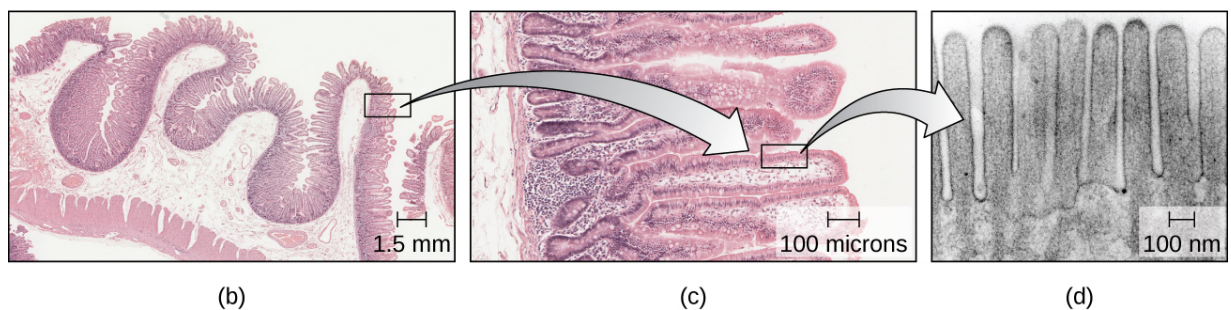
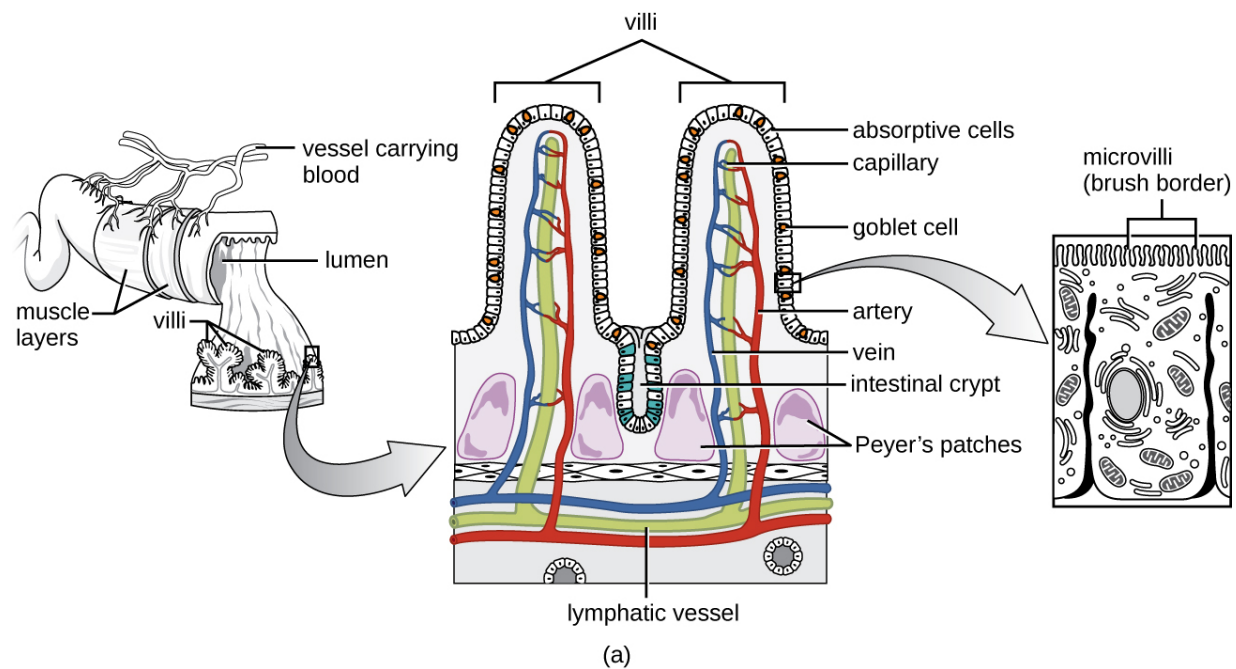
Note:

- What factors make the mouth inhospitable for certain microbes?

Anatomy and Normal Microbiota of the GI Tract

As food leaves the oral cavity, it travels through the pharynx, or the back of the throat, and moves into the esophagus, which carries the food from the pharynx to the stomach without adding any additional digestive enzymes. The stomach produces mucus to protect its lining, as well as digestive enzymes and acid to break down food. Partially digested food then leaves the stomach through the pyloric sphincter, reaching the first part of the

small intestine called the duodenum. Pancreatic juice, which includes enzymes and bicarbonate ions, is released into the small intestine to neutralize the acidic material from the stomach and to assist in digestion. Bile, produced by the liver but stored in the gallbladder, is also released into the small intestine to emulsify fats so that they can travel in the watery environment of the small intestine. Digestion continues in the small intestine, where the majority of nutrients contained in the food are absorbed. Simple columnar epithelial cells called enterocytes line the lumen surface of the small intestinal folds called villi. Each enterocyte has smaller microvilli (cytoplasmic membrane extensions) on the cellular apical surface that increase the surface area to allow more absorption of nutrients to occur ([link]).



(a) The structure of the wall of the small intestine allows for the majority of nutrient absorption in the body. (b) Villi are folds in the surface of the small intestine. Microvilli are cytoplasmic extensions on individual cells that increase the surface area for absorption. (c) A light micrograph shows the shape of the villi. (d) An electron micrograph shows the shape of the microvilli. (credit b, c, d: Modification of micrographs provided by the Regents of University of Michigan Medical School © 2012)

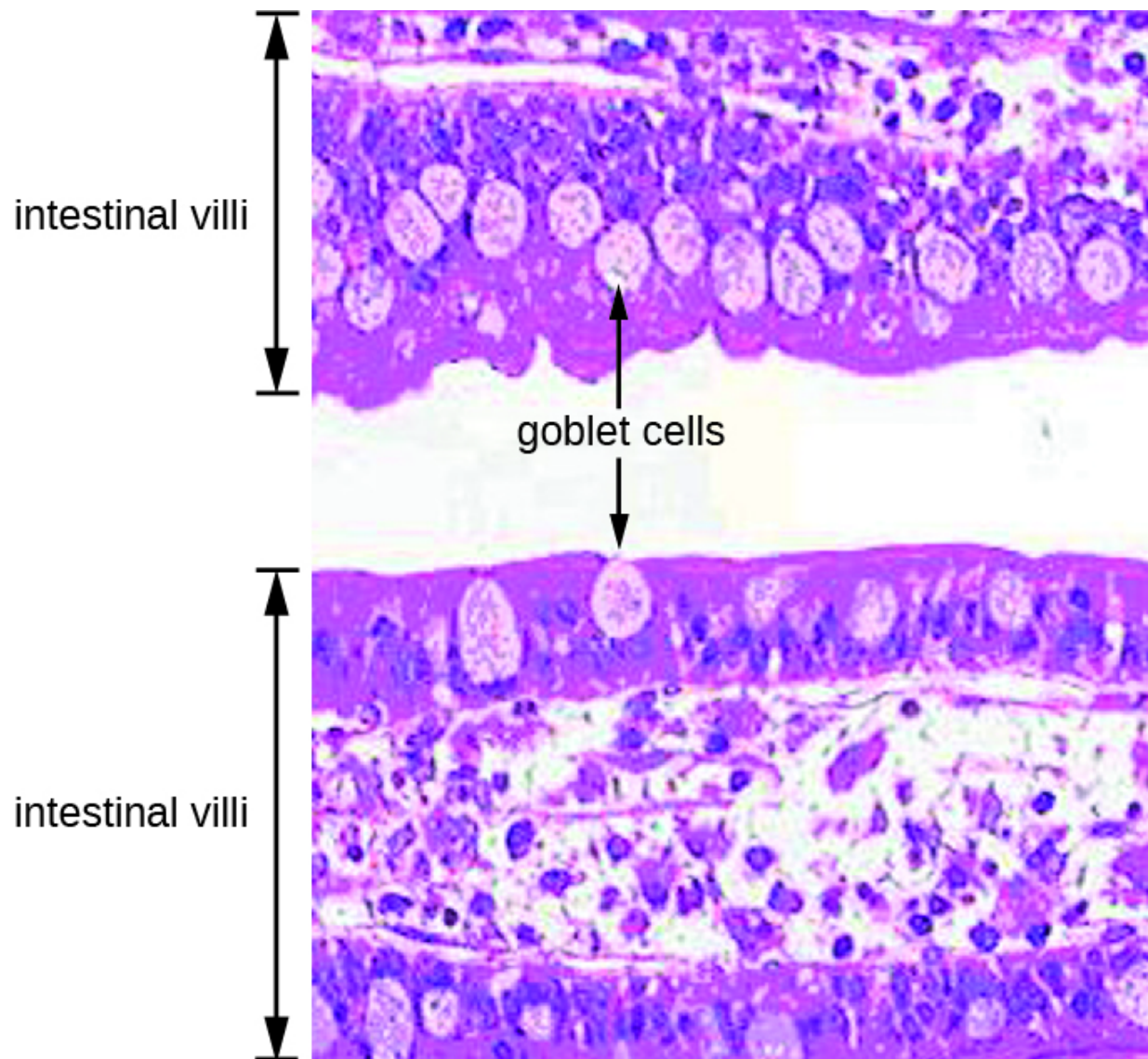
Digested food leaves the small intestine and moves into the large intestine, or colon, where there is a more diverse microbiota. Near this junction, there is a small pouch in the large intestine called the cecum, which attaches to the appendix. Further digestion occurs throughout the colon and water is reabsorbed, then waste is excreted through the rectum, the last section of the colon, and out of the body through the anus ([\[link\]](#)).

The environment of most of the GI tract is harsh, which serves two purposes: digestion and immunity. The stomach is an extremely acidic environment (pH 1.5–3.5) due to the gastric juices that break down food and kill many ingested microbes; this helps prevent infection from pathogens. The environment in the small intestine is less harsh and is able to support microbial communities. Microorganisms present in the small intestine can include lactobacilli, diptherioids and the fungus *Candida*. On the other hand, the large intestine (colon) contains a diverse and abundant microbiota that is important for normal function. These microbes include *Bacterioidetes* (especially the genera *Bacteroides* and *Prevotella*) and *Firmicutes* (especially members of the genus *Clostridium*). Methanogenic archaea and some fungi are also present, among many other species of bacteria. These microbes all aid in digestion and contribute to the production of feces, the waste excreted from the digestive tract, and flatus, the gas produced from microbial fermentation of undigested food. They can also produce valuable nutrients. For example, lactic acid bacteria such as bifidobacteria can synthesize vitamins, such as vitamin B12, folate, and riboflavin, that humans cannot synthesize themselves. *E. coli* found in the

intestine can also break down food and help the body produce vitamin K, which is important for blood coagulation.

The GI tract has several other methods of reducing the risk of infection by pathogens. Small aggregates of underlying lymphoid tissue in the ileum, called **Peyer's patches** ([\[link\]](#)), detect pathogens in the intestines via microfold (M) cells, which transfer antigens from the lumen of the intestine to the lymphocytes on Peyer's patches to induce an immune response. The Peyer's patches then secrete IgA and other pathogen-specific antibodies into the intestinal lumen to help keep intestinal microbes at safe levels. Goblet cells, which are modified simple columnar epithelial cells, also line the GI tract ([\[link\]](#)). Goblet cells secrete a gel-forming mucin, which is the major component of mucus. The production of a protective layer of mucus helps reduce the risk of pathogens reaching deeper tissues.

The constant movement of materials through the gastrointestinal tract also helps to move transient pathogens out of the body. In fact, feces are composed of approximately 25% microbes, 25% sloughed epithelial cells, 25% mucus, and 25% digested or undigested food. Finally, the normal microbiota provides an additional barrier to infection via a variety of mechanisms. For example, these organisms outcompete potential pathogens for space and nutrients within the intestine. This is known as competitive exclusion. Members of the microbiota may also secrete protein toxins known as bacteriocins that are able to bind to specific receptors on the surface of susceptible bacteria.



A magnified image of intestinal villi in the GI tract shows goblet cells. These cells are important in producing a protective layer of mucus.

Note:

- Compare and contrast the microbiota of the small and large intestines.

General Signs and Symptoms of Oral and GI Disease

Despite numerous defense mechanisms that protect against infection, all parts of the digestive tract can become sites of infection or intoxication. The term food poisoning is sometimes used as a catch-all for GI infections and intoxications, but not all forms of GI disease originate with foodborne pathogens or toxins.

In the mouth, fermentation by anaerobic microbes produces acids that damage the teeth and gums. This can lead to tooth decay, cavities, and **periodontal disease**, a condition characterized by chronic inflammation and erosion of the gums. Additionally, some pathogens can cause infections of the mucosa, glands, and other structures in the mouth, resulting in inflammation, sores, cankers, and other lesions. An open sore in the mouth or GI tract is typically called an **ulcer**.

Infections and intoxications of the lower GI tract often produce symptoms such as nausea, vomiting, diarrhea, aches, and fever. In some cases, vomiting and diarrhea may cause severe dehydration and other complications that can become serious or fatal. Various clinical terms are used to describe gastrointestinal symptoms. For example, **gastritis** is an inflammation of the stomach lining that results in swelling and **enteritis** refers to inflammation of the intestinal mucosa. When the inflammation involves both the stomach lining and the intestinal lining, the condition is called **gastroenteritis**. Inflammation of the liver is called **hepatitis**. Inflammation of the colon, called **colitis**, commonly occurs in cases of food intoxication. Because an inflamed colon does not reabsorb water as effectively as it normally does, stools become watery, causing diarrhea. Damage to the epithelial cells of the colon can also cause bleeding and excess mucus to appear in watery stools, a condition called **dysentery**.

Note:

- List possible causes and signs and symptoms of food poisoning.

Key Concepts and Summary

- The digestive tract, consisting of the oral cavity, pharynx, esophagus, stomach, small intestine, and large intestine, has a normal microbiota that is important for health.
- The constant movement of materials through the gastrointestinal canal, the protective layer of mucus, the normal microbiota, and the harsh chemical environment in the stomach and small intestine help to prevent colonization by pathogens.
- Infections or microbial toxins in the oral cavity can cause **tooth decay**, **periodontal disease**, and various types of **ulcers**.
- Infections and intoxications of the gastrointestinal tract can cause general symptoms such as nausea, vomiting, diarrhea, and fever. Localized inflammation of the GI tract can result in **gastritis**, **enteritis**, **gastroenteritis**, **hepatitis**, or **colitis**, and damage to epithelial cells of the colon can lead to **dysentery**.
- **Foodborne illness** refers to infections or intoxications that originate with pathogens or toxins ingested in contaminated food or water.

Multiple Choice

Exercise:

Problem:

Which of the following is NOT a way the normal microbiota of the intestine helps to prevent infection?

- a. It produces acids that lower the pH of the stomach.
- b. It speeds up the process by which microbes are flushed from the digestive tract.
- c. It consumes food and occupies space, outcompeting potential pathogens.
- d. It generates large quantities of oxygen that kill anaerobic pathogens.

Solution:

D

Exercise:

Problem: What types of microbes live in the intestines?

- a. Diverse species of bacteria, archaea, and fungi, especially *Bacteroides* and *Firmicutes* bacteria
- b. A narrow range of bacteria, especially *Firmicutes*
- c. A narrow range of bacteria and fungi, especially *Bacteroides*
- d. Archaea and fungi only

Solution:

A

Fill in the Blank

Exercise:

Problem:

The part of the gastrointestinal tract with the largest natural microbiota is the _____.

Solution:

Large intestine or colon

Short Answer

Exercise:

Problem:

How does the diarrhea caused by dysentery differ from other types of diarrhea?

Microbial Diseases of the Mouth and Oral Cavity

LEARNING OBJECTIVES

- Explain the role of microbial activity in diseases of the mouth and oral cavity
- Compare the major characteristics of specific oral diseases and infections

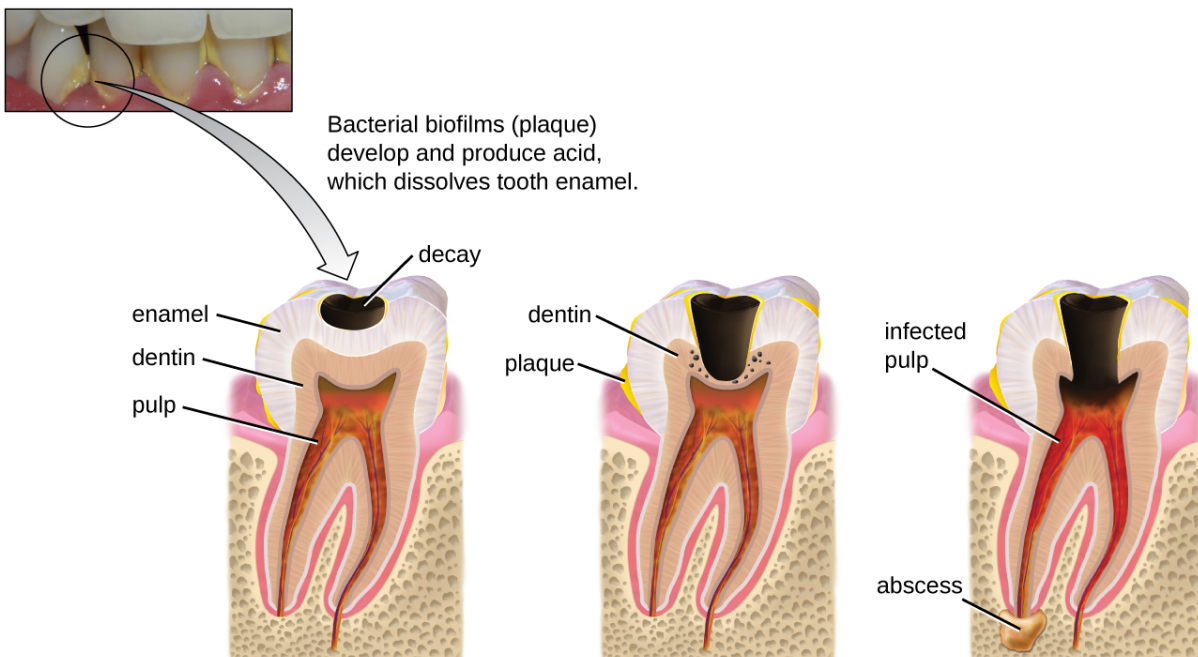
Despite the presence of saliva and the mechanical forces of chewing and eating, some microbes thrive in the mouth. These microbes can cause damage to the teeth and can cause infections that have the potential to spread beyond the mouth and sometimes throughout the body.

Dental Caries

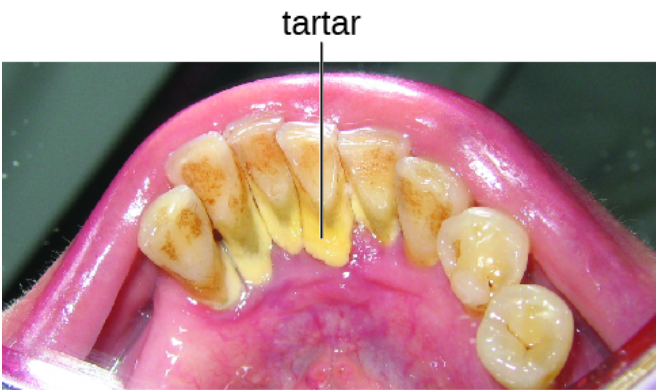
Cavities of the teeth, known clinically as **dental caries**, are microbial lesions that cause damage to the teeth. Over time, the lesion can grow through the outer enamel layer to infect the underlying dentin or even the innermost pulp. If dental caries are not treated, the infection can become an abscess that spreads to the deeper tissues of the teeth, near the roots, or to the bloodstream.

Tooth decay results from the metabolic activity of microbes that live on the teeth. A layer of proteins and carbohydrates forms when clean teeth come into contact with saliva. Microbes are attracted to this food source and form a biofilm called plaque. The most important cariogenic species in these biofilms is *Streptococcus mutans*. When sucrose, a disaccharide sugar from food, is broken down by bacteria in the mouth, glucose and fructose are

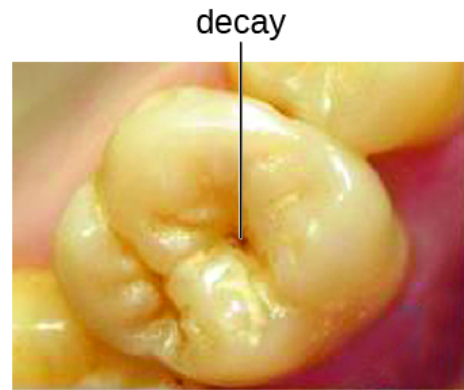
produced. The glucose is used to make dextran, which is part of the extracellular matrix of the biofilm. Fructose is fermented, producing organic acids such as lactic acid. These acids dissolve the minerals of the tooth, including enamel, even though it is the hardest material in the body. The acids work even more quickly on exposed dentin ([link](#)). Over time, the plaque biofilm can become thick and eventually calcify. When a heavy plaque deposit becomes hardened in this way, it is called **tartar** or **dental calculus** ([link](#)). These substantial plaque biofilms can include a variety of bacterial species, including *Streptococcus* and *Actinomyces* species.



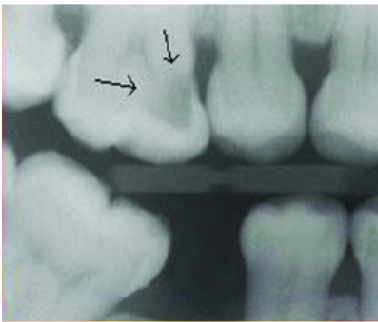
Tooth decay occurs in stages. When bacterial biofilms (plaque) develop on teeth, the acids produced gradually dissolve the enamel, followed by the dentin. Eventually, if left untreated, the lesion may reach the pulp and cause an abscess. (credit: modification of work by “BruceBlaus”/Wikimedia Commons)



(a)



(b)



(c)



(d)



(e)

(a) Tartar (dental calculus) is visible at the bases of these teeth. The darker deposits higher on the crowns are staining. (b) This tooth shows only a small amount of visible decay. (c) An X-ray of the same tooth shows that there is a dark area representing more decay inside the tooth. (d) Removal of a portion of the crown reveals the area of damage. (e) All of the cavity must be removed before filling. (credit: modification of work by “DRosenbach”/Wikimedia Commons)

Some tooth decay is visible from the outside, but it is not always possible to see all decay or the extent of the decay. X-ray imaging is used to produce radiographs that can be studied to look for deeper decay and damage to the root or bone ([link](#)). If not detected, the decay can reach the pulp or even spread to the bloodstream. Painful abscesses can develop.

To prevent tooth decay, prophylactic treatment and good hygiene are important. Regular tooth brushing and flossing physically removes microbes and combats microbial growth and biofilm formation. Toothpaste contains fluoride, which becomes incorporated into the hydroxyapatite of tooth enamel, protecting it against acidity caused by fermentation of mouth microbiota. Fluoride is also bacteriostatic, thus slowing enamel degradation. Antiseptic mouthwashes commonly contain plant-derived phenolics like thymol and eucalyptol and/or heavy metals like zinc chloride (see [Using Chemicals to Control Microorganisms](#)). Phenolics tend to be stable and persistent on surfaces, and they act through denaturing proteins and disrupting membranes.

Regular dental cleanings allow for the detection of decay at early stages and the removal of tartar. They may also help to draw attention to other concerns, such as damage to the enamel from acidic drinks. Reducing sugar consumption may help prevent damage that results from the microbial fermentation of sugars. Additionally, sugarless candies or gum with sugar alcohols (such as xylitol) can reduce the production of acids because these are fermented to nonacidic compounds (although excess consumption may lead to gastrointestinal distress). Fluoride treatment or ingesting fluoridated water strengthens the minerals in teeth and reduces the incidence of dental caries.

If caries develop, prompt treatment prevents worsening. Smaller areas of decay can be drilled to remove affected tissue and then filled. If the pulp is affected, then a root canal may be needed to completely remove the infected tissues to avoid continued spread of the infection, which could lead to painful abscesses.

Note:

- Name some ways that microbes contribute to tooth decay.
- What is the most important cariogenic species of bacteria?

Periodontal Disease

In addition to damage to the teeth themselves, the surrounding structures can be affected by microbes. Periodontal disease is the result of infections that lead to inflammation and tissue damage in the structures surrounding the teeth. The progression from mild to severe periodontal disease is generally reversible and preventable with good oral hygiene.

Inflammation of the gums that can lead to irritation and bleeding is called **gingivitis**. When plaque accumulates on the teeth, bacteria colonize the gingival space. As this space becomes increasingly blocked, the environment becomes anaerobic. This allows a wide variety of microbes to colonize, including *Porphyromonas*, *Streptococcus*, and *Actinomyces*. The bacterial products, which include lipopolysaccharide (LPS), proteases, lipoteichoic acids, and others, cause inflammation and gum damage ([\[link\]](#)). It is possible that methanogenic archaeans (including *Methanobrevibacter oralis* and other *Methanobrevibacter* species) also contribute to disease progression as some species have been identified in patients with periodontal disease, but this has proven difficult to study.[\[footnote\]](#) [\[footnote\]](#) [\[footnote\]](#) Gingivitis is diagnosed by visual inspection, including measuring pockets in the gums, and X-rays, and is usually treated using good dental hygiene and professional dental cleaning, with antibiotics reserved for severe cases.

Hans-Peter Horz and Georg Conrads. “Methanogenic Archaea and Oral Infections—Ways to Unravel the Black Box.” *Journal of Oral Microbiology* 3(2011). doi: 10.3402/jom.v3i0.5940.

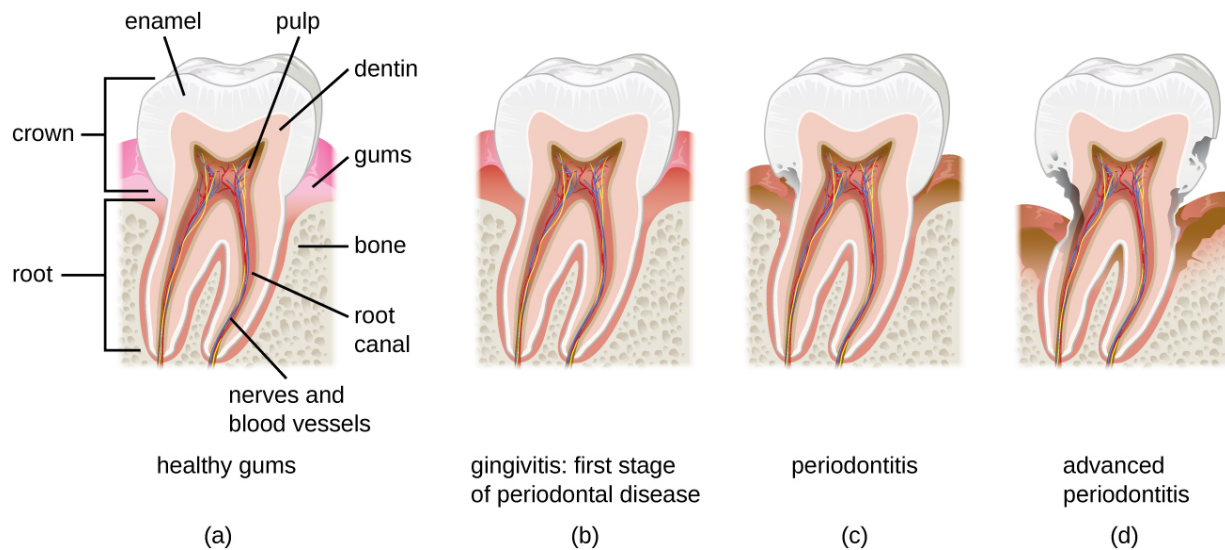
Hiroshi Maeda, Kimito Hirai, Junji Mineshiba, Tadashi Yamamoto, Susumu Koeguchi, and Shogo Takashiba. “Medical Microbiological Approach to Archaea in Oral Infectious Diseases.” *Japanese Dental Science Review* 49: 2, p. 72–78.

Paul W. Lepp, Mary M. Brinig, Cleber C. Ouverney, Katherine Palm, Gary C. Armitage, and David A. Relman. “Methanogenic Archaea and Human Periodontal Disease.” *Proceedings of the National Academy of Sciences of the United States of America* 101 (2003): 16, pp. 6176–6181. doi: 10.1073/pnas.0308766101.



Redness and irritation of the gums are evidence of gingivitis.

Over time, chronic gingivitis can develop into the more serious condition of **periodontitis** ([link](#)). When this happens, the gums recede and expose parts of the tooth below the crown. This newly exposed area is relatively unprotected, so bacteria can grow on it and spread underneath the enamel of the crown and cause cavities. Bacteria in the gingival space can also erode the cementum, which helps to hold the teeth in place. If not treated, erosion of cementum can lead to the movement or loss of teeth. The bones of the jaw can even erode if the infection spreads. This condition can be associated with bleeding and halitosis (bad breath). Cleaning and appropriate dental hygiene may be sufficient to treat periodontitis. However, in cases of severe periodontitis, an antibiotic may be given. Antibiotics may be given in pill form or applied directly to the gum (local treatment). Antibiotics given can include tetracycline, doxycycline, macrolides or β -lactams. Because periodontitis can be caused by a mix of microbes, a combination of antibiotics may be given.



(a) Healthy gums hold the teeth firmly and do not bleed. (b) Gingivitis is the first stage of periodontal disease. Microbial infection causes gums to become inflamed and irritated, with occasional bleeding. (c) In periodontitis, gums recede and expose parts of the tooth normally covered. (d) In advanced periodontitis, the infection spreads to ligaments and bone tissue supporting the teeth. Tooth loss may occur, or teeth may need to be surgically removed. (credit: modification of work by “BruceBlaus”/Wikimedia Commons)

Trench Mouth

When certain bacteria, such as *Prevotella intermedia*, *Fusobacterium* species, and *Treponema vincentii*, are involved and periodontal disease progresses, **acute necrotizing ulcerative gingivitis** or **trench mouth**, also called Vincent's disease, can develop. This is severe periodontitis characterized by erosion of the gums, ulcers, substantial pain with chewing, and halitosis ([link](#)) that can be diagnosed by visual examination and X-rays. In countries with good medical and dental care, it is most common in individuals with weakened immune systems, such as patients with AIDS. In addition to cleaning and pain medication, patients may be prescribed

antibiotics such as amoxicillin, amoxicillin clavulanate, clindamycin, or doxycycline.



These inflamed, eroded gums are an example of a mild case of acute necrotizing ulcerative gingivitis, also known as trench mouth. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- How does gingivitis progress to periodontitis?

Note:**Healthy Mouth, Healthy Body**

Good oral health promotes good overall health, and the reverse is also true. Poor oral health can lead to difficulty eating, which can cause malnutrition. Painful or loose teeth can also cause a person to avoid certain foods or eat less. Malnutrition due to dental problems is of greatest concern for the elderly, for whom it can worsen other health conditions and contribute to mortality. Individuals who have serious illnesses, especially AIDS, are also at increased risk of malnutrition from dental problems.

Additionally, poor oral health can contribute to the development of disease. Increased bacterial growth in the mouth can cause inflammation and infection in other parts of the body. For example, *Streptococcus* in the mouth, the main contributor to biofilms on teeth, tartar, and dental caries, can spread throughout the body when there is damage to the tissues inside the mouth, as can happen during dental work. *S. mutans* produces a surface adhesin known as P1, which binds to salivary agglutinin on the surface of the tooth. P1 can also bind to extracellular matrix proteins including fibronectin and collagen. When *Streptococcus* enters the bloodstream as a result of tooth brushing or dental cleaning, it causes inflammation that can lead to the accumulation of plaque in the arteries and contribute to the development of atherosclerosis, a condition associated with cardiovascular disease, heart attack, and stroke. In some cases, bacteria that spread through the blood vessels can lodge in the heart and cause endocarditis (an example of a focal infection).

Oral Infections

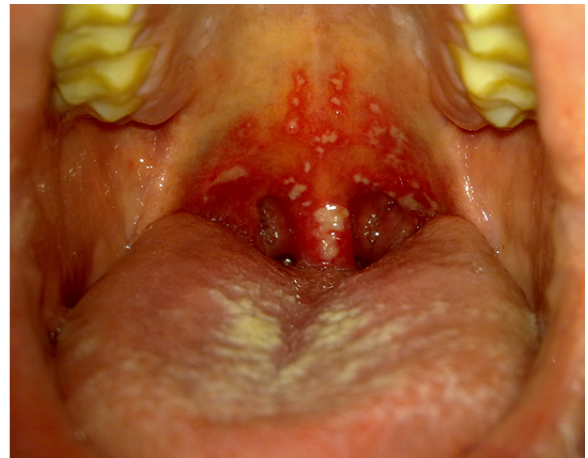
As noted earlier, normal oral microbiota can cause dental and periodontal infections. However, there are number of other infections that can manifest in the oral cavity when other microbes are present.

Herpetic Gingivostomatitis

As described in [Viral Infections of the Skin and Eyes](#), infections by herpes simplex virus type 1 (HSV-1) frequently manifest as oral herpes, also called acute herpes labialis and characterized by cold sores on the lips, mouth, or gums. HSV-1 can also cause acute **herpetic gingivostomatitis**, a condition that results in ulcers of the mucous membranes inside the mouth ([link](#)). Herpetic gingivostomatitis is normally self-limiting except in immunocompromised patients. Like oral herpes, the infection is generally diagnosed through clinical examination, but cultures or biopsies may be obtained if other signs or symptoms suggest the possibility of a different causative agent. If treatment is needed, mouthwashes or antiviral medications such as acyclovir, famciclovir, or valacyclovir may be used.



(a)



(b)

(a) This cold sore is caused by infection with herpes simplex virus type 1 (HSV-1). (b) HSV-1 can also cause acute herpetic gingivostomatitis. (credit b: modification of work by Klaus D. Peter)

Oral Thrush

The yeast *Candida* is part of the normal human microbiota, but overgrowths, especially of *Candida albicans*, can lead to infections in

several parts of the body. When *Candida* infection develops in the oral cavity, it is called **oral thrush**. Oral thrush is most common in infants because they do not yet have well developed immune systems and have not acquired the robust normal microbiota that keeps *Candida* in check in adults. Oral thrush is also common in immunodeficient patients and is a common infection in patients with AIDS.

Oral thrush is characterized by the appearance of white patches and pseudomembranes in the mouth ([\[link\]](#)) and can be associated with bleeding. The infection may be treated topically with nystatin or clotrimazole oral suspensions, although systemic treatment is sometimes needed. In serious cases, systemic azoles such as fluconazole or itraconazole (for strains resistant to fluconazole), may be used. Amphotericin B can also be used if the infection is severe or if the *Candida* species is azole-resistant.



Overgrowth of *Candida* in the mouth is called thrush. It often appears

as white patches. (credit: modification of work by Centers for Disease Control and Prevention)

Mumps

The viral disease **mumps** is an infection of the parotid glands, the largest of the three pairs of salivary glands ([\[link\]](#)). The causative agent is mumps virus (MuV), a paramyxovirus with an envelope that has hemagglutinin and neuraminidase spikes. A fusion protein located on the surface of the envelope helps to fuse the viral envelope to the host cell plasma membrane.

Mumps virus is transmitted through respiratory droplets or through contact with contaminated saliva, making it quite contagious so that it can lead easily to epidemics. It causes fever, muscle pain, headache, pain with chewing, loss of appetite, fatigue, and weakness. There is swelling of the salivary glands and associated pain ([\[link\]](#)). The virus can enter the bloodstream (viremia), allowing it to spread to the organs and the central nervous system. The infection ranges from subclinical cases to cases with serious complications, such as encephalitis, meningitis, and deafness. Inflammation of the pancreas, testes, ovaries, and breasts may also occur and cause permanent damage to those organs; despite these complications, a mumps infection rarely cause sterility.

Mumps can be recognized based on clinical signs and symptoms, and a diagnosis can be confirmed with laboratory testing. The virus can be identified using culture or molecular techniques such as RT-PCR. Serologic tests are also available, especially enzyme immunoassays that detect antibodies. There is no specific treatment for mumps, so supportive therapies are used. The most effective way to avoid infection is through vaccination. Although mumps used to be a common childhood disease, it is now rare in the United States due to vaccination with the measles, mumps, and rubella (MMR) vaccine.



This child shows the characteristic parotid swelling associated with mumps. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- Compare and contrast the signs and symptoms of herpetic gingivostomatitis, oral thrush, and mumps.

Note:**Oral Infections**

Infections of the mouth and oral cavity can be caused by a variety of pathogens, including bacteria, viruses, and fungi. Many of these infections only affect the mouth, but some can spread and become systemic infections. [\[link\]](#) summarizes the main characteristics of common oral infections.

Oral Infections					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Dental caries	<i>Streptococcus mutans</i>	Discoloration, softening, cavities in teeth	Non-transmissible; caused by bacteria of the normal oral microbiota	Visual examinations, X-rays	Oral antiseptics (e.g., Listerine)
Gingivitis and periodontitis	<i>Porphyromonas</i> , <i>Streptococcus</i> , <i>Actinomyces</i>	Inflammation and erosion of gums, bleeding, halitosis; erosion of cementum, leading to tooth loss in advanced infections	Non-transmissible; caused by bacteria of the normal oral microbiota	Visual examination, X-rays, measuring pockets in gums	Tetracycline, doxycycline, macrolides or beta-lactams. Mixture of antibiotics may be given.
Herpetic gingivostomatitis	Herpes simplex virus type 1 (HSV-1)	Lesions in mucous membranes of mouth	Contact with saliva or lesions of an infected person	Culture or biopsy	Acyclovir, famcyclovir, valacyclovir
Mumps	Mumps virus (a paramyxovirus)	Swelling of parotid glands, fever, headache, muscle pain, weakness, fatigue, loss of appetite, pain while chewing; in serious cases, encephalitis, meningitis, and inflammation of testes, ovaries, and breasts	Contact with saliva or respiratory droplets of an infected person	Virus culture or serologic tests for antibodies, enzyme immunoassay, RT-PCR	None for treatment; MMR vaccine for prevention
Oral thrush	<i>Candida albicans</i> , other <i>Candida</i> spp.	White patches and pseudomembranes in mouth, may cause bleeding	Non-transmissible; caused by overgrowth of <i>Candida</i> spp. in the normal oral microbiota; primarily affects infants and the immuno-compromised	Microscopic analysis of oral samples	Clotrimazole, nystatin, fluconazole, or itraconazole; amphotericin B in severe cases
Trench mouth (acute necrotizing ulcerative gingivitis)	<i>Prevotella intermedia</i> , <i>Fusobacterium</i> species, <i>Treponema vincentii</i> , others	Erosion of gums, ulcers, substantial pain with chewing, halitosis	Non-transmissible; caused by members of the normal oral microbiota	Visual examinations, X-rays	Amoxicillin, amoxicillin clavulanate, clindamycin, or doxycycline

Key Concepts and Summary

- **Dental caries, tartar, and gingivitis** are caused by overgrowth of oral bacteria, usually *Streptococcus* and *Actinomyces* species, as a result of insufficient dental hygiene.
- Gingivitis can worsen, allowing *Porphyromonas*, *Streptococcus*, and *Actinomyces* species to spread and cause **periodontitis**. When *Prevotella intermedia*, *Fusobacterium* species, and *Treponema vincentii* are involved, it can lead to **acute necrotizing ulcerative gingivitis**.
- The herpes simplex virus type 1 can cause lesions of the mouth and throat called **herpetic gingivostomatitis**.
- Other infections of the mouth include **oral thrush**, a fungal infection caused by overgrowth of *Candida* yeast, and **mumps**, a viral infection of the salivary glands caused by the mumps virus, a paramyxovirus.

Multiple Choice

Exercise:

Problem:

What pathogen is the most important contributor to biofilms in plaque?

- Staphylococcus aureus*
- Streptococcus mutans*
- Escherichia coli*
- Clostridium difficile*

Solution:

B

Exercise:

Problem: What type of organism causes thrush?

- a bacterium
- a virus
- a fungus

d. a protozoan

Solution:

C

Exercise:

Problem:

In mumps, what glands swell to produce the disease's characteristic appearance?

- a. the sublingual glands
- b. the gastric glands
- c. the parotid glands
- d. the submandibular glands

Solution:

C

Exercise:

Problem: Which of the following is true of HSV-1?

- a. It causes oral thrush in immunocompromised patients.
- b. Infection is generally self-limiting.
- c. It is a bacterium.
- d. It is usually treated with amoxicillin.

Solution:

B

Fill in the Blank

Exercise:**Problem:**

When plaque becomes heavy and hardened, it is called dental calculus or _____.

Solution:

tartar

Short Answer**Exercise:**

Problem: Why do sugary foods promote dental caries?

Bacterial Infections of the Gastrointestinal Tract

LEARNING OBJECTIVES

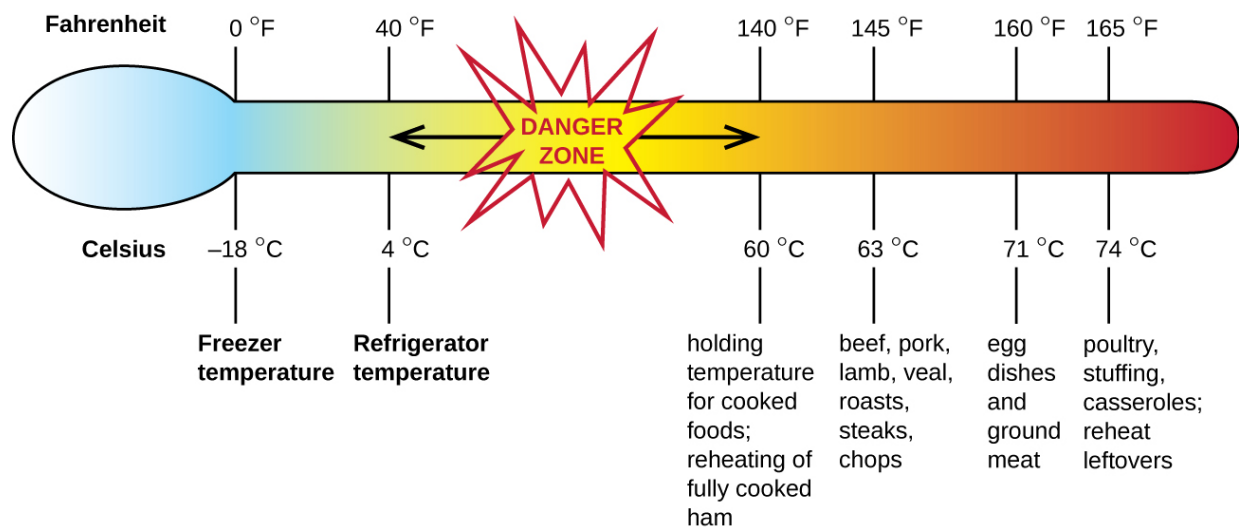
- Identify the most common bacteria that can cause infections of the GI tract
- Compare the major characteristics of specific bacterial diseases affecting the GI tract

A wide range of gastrointestinal diseases are caused by bacterial contamination of food. Recall that **foodborne disease** can arise from either infection or intoxication. In both cases, bacterial toxins are typically responsible for producing disease signs and symptoms. The distinction lies in where the toxins are produced. In an infection, the microbial agent is ingested, colonizes the gut, and then produces toxins that damage host cells. In an intoxication, bacteria produce toxins in the food before it is ingested. In either case, the toxins cause damage to the cells lining the gastrointestinal tract, typically the colon. This leads to the common signs and symptoms of diarrhea or watery stool and abdominal cramps, or the more severe dysentery. Symptoms of foodborne diseases also often include nausea and vomiting, which are mechanisms the body uses to expel the toxic materials.

Most bacterial gastrointestinal illness is short-lived and self-limiting; however, loss of fluids due to severe diarrheal illness can lead to dehydration that can, in some cases, be fatal without proper treatment. Oral rehydration therapy with electrolyte solutions is an essential aspect of treatment for most patients with GI disease, especially in children and infants.

Staphylococcal Food Poisoning

Staphylococcal food poisoning is one form of food intoxication. When *Staphylococcus aureus* grows in food, it may produce enterotoxins that, when ingested, can cause symptoms such as nausea, diarrhea, cramping, and vomiting within one to six hours. In some severe cases, it may cause headache, dehydration, and changes in blood pressure and heart rate. Signs and symptoms resolve within 24 to 48 hours. *S. aureus* is often associated with a variety of raw or undercooked and cooked foods including meat (e.g., canned meat, ham, and sausages) and dairy products (e.g., cheeses, milk, and butter). It is also commonly found on hands and can be transmitted to prepared foods through poor hygiene, including poor handwashing and the use of contaminated food preparation surfaces, such as cutting boards. The greatest risk is for food left at a temperature below 60 °C (140 °F), which allows the bacteria to grow. Cooked foods should generally be reheated to at least 60 °C (140 °F) for safety and most raw meats should be cooked to even higher internal temperatures ([link](#)).



This figure indicates safe internal temperatures associated with the refrigeration, cooking, and reheating of different foods. Temperatures above refrigeration and below the minimum cooking temperature may allow for microbial growth, increasing the likelihood of foodborne disease.
(credit: modification of work by USDA)

There are at least 21 *Staphylococcal* enterotoxins and *Staphylococcal* enterotoxin-like toxins that can cause food intoxication. The enterotoxins are proteins that are resistant to low pH, allowing them to pass through the stomach. They are heat stable and are not destroyed by boiling at 100 °C. Even though the bacterium itself may be killed, the enterotoxins alone can cause vomiting and diarrhea, although the mechanisms are not fully understood. At least some of the symptoms may be caused by the enterotoxin functioning as a superantigen and provoking a strong immune response by activating T cell proliferation.

The rapid onset of signs and symptoms helps to diagnose this foodborne illness. Because the bacterium does not need to be present for the toxin to cause symptoms, diagnosis is confirmed by identifying the toxin in a food sample or in biological specimens (feces or vomitus) from the patient. Serological techniques, including ELISA, can also be used to identify the toxin in food samples.

The condition generally resolves relatively quickly, within 24 hours, without treatment. In some cases, supportive treatment in a hospital may be needed.

Note:

- How can *S. aureus* cause food intoxication?

Shigellosis (Bacillary Dysentery)

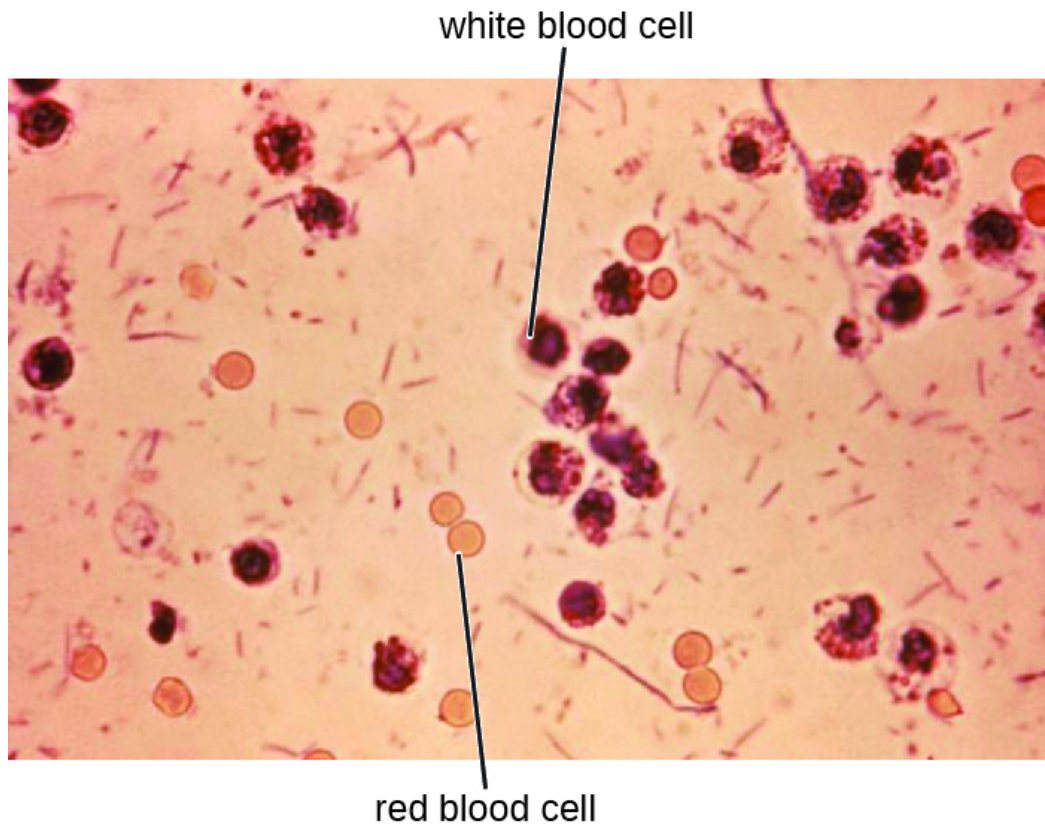
When gastrointestinal illness is associated with the rod-shaped, gram-negative bacterium *Shigella*, it is called **bacillary dysentery**, or **shigellosis**. Infections can be caused by *S. dysenteriae*, *S. flexneri*, *S. boydii*, and/or *S. sonnei* that colonize the GI tract. Shigellosis can be spread from hand to mouth or through contaminated food and water. Most commonly, it is transmitted through the fecal-oral route.

Shigella bacteria invade intestinal epithelial cells. When taken into a phagosome, they can escape and then live within the cytoplasm of the cell or move to adjacent cells. As the organisms multiply, the epithelium and structures with M cells of the Peyer's patches in the intestine may become ulcerated and cause loss of fluid. Stomach cramps, fever, and watery diarrhea that may also contain pus, mucus, and/or blood often develop. More severe cases may result in ulceration of the mucosa, dehydration, and rectal bleeding. Additionally, patients may later develop hemolytic uremic syndrome (HUS), a serious condition in which damaged blood cells build up in the kidneys and may cause kidney failure, or reactive arthritis, a condition in which arthritis develops in multiple joints following infection. Patients may also develop chronic post-infection irritable bowel syndrome (IBS).

S. dysenteriae type 1 is able to produce Shiga toxin, which targets the endothelial cells of small blood vessels in the small and large intestine by binding to a glycosphingolipid. Once inside the endothelial cells, the toxin targets the large ribosomal subunit, thus affecting protein synthesis of these cells. Hemorrhaging and lesions in the colon can result. The toxin can target the kidney's glomerulus, the blood vessels where filtration of blood in the kidney begins, thus resulting in HUS.

Stool samples, which should be processed promptly, are analyzed using serological or molecular techniques. One common method is to perform immunoassays for *S. dysenteriae*. (Other methods that can be used to identify *Shigella* include API test strips, Enterotube systems, or PCR testing. The presence of white blood cells and blood in fecal samples occurs in about 70% of patients^[footnote] ([link](#))). Severe cases may require antibiotics such as ciprofloxacin and azithromycin, but these must be carefully prescribed because resistance is increasingly common.

Jaya Sureshababu. "Shigella Infection Workup." *Medscape*. Updated Jun 28, 2016.
<http://emedicine.medscape.com/article/968773-workup>.



Red and white blood cells can be seen in this micrograph of a stool sample from a patient with shigellosis.

Note:

- Compare and contrast *Shigella* infections and intoxications.

Salmonellosis

Salmonella gastroenteritis, also called **salmonellosis**, is caused by the rod-shaped, gram-negative bacterium *Salmonella*. Two species, *S. enterica* and *S. bongori*, cause disease in humans, but *S. enterica* is the most common. The most common serotypes of *S. enterica* are Enteritidis and Typhi. We will discuss typhoid fever caused by serotypes Typhi and Paratyphi A separately. Here, we will focus on salmonellosis caused by other serotypes.

Salmonella is a part of the normal intestinal microbiota of many individuals. However, salmonellosis is caused by exogenous agents, and infection can occur depending on the serotype, size of the inoculum, and overall health of the host. Infection is caused by ingestion of contaminated food,

handling of eggshells, or exposure to certain animals. *Salmonella* is part of poultry's microbiota, so exposure to raw eggs and raw poultry can increase the risk of infection. Handwashing and cooking foods thoroughly greatly reduce the risk of transmission. *Salmonella* bacteria can survive freezing for extended periods but cannot survive high temperatures.

Once the bacteria are ingested, they multiply within the intestines and penetrate the epithelial mucosal cells via M cells where they continue to grow ([link](#)). They trigger inflammatory processes and the hypersecretion of fluids. Once inside the body, they can persist inside the phagosomes of macrophages. *Salmonella* can cross the epithelial cell membrane and enter the bloodstream and lymphatic system. Some strains of *Salmonella* also produce an enterotoxin that can cause an intoxication.

Infected individuals develop fever, nausea, abdominal cramps, vomiting, headache, and diarrhea. These signs and symptoms generally last a few days to a week. According to the Centers for Disease Control and Prevention (CDC), there are 1,000,000 cases annually, with 380 deaths each year. [\[footnote\]](#) However, because the disease is usually self-limiting, many cases are not reported to doctors and the overall incidence may be underreported. Diagnosis involves culture followed by serotyping and DNA fingerprinting if needed. Positive results are reported to the CDC. When an unusual serotype is detected, samples are sent to the CDC for further analysis. Serotyping is important for determining treatment. Oral rehydration therapy is commonly used. Antibiotics are only recommended for serious cases. When antibiotics are needed, as in immunocompromised patients, fluoroquinolones, third-generation cephalosporins, and ampicillin are recommended. Antibiotic resistance is a serious concern.

Centers for Disease Control and Prevention. *Salmonella*. Updated August 25, 2016.
<https://www.cdc.gov/salmonella>.



Salmonella entering an intestinal epithelial cell by reorganizing the host cell's cytoskeleton via the trigger mechanism. (credit: modification of work by National Institutes for Health)

Typhoid Fever

Certain serotypes of *S. enterica*, primarily serotype Typhi (*S. typhi*) but also Paratyphi, cause a more severe type of salmonellosis called **typhoid fever**. This serious illness, which has an untreated mortality rate of 10%, causes high fever, body aches, headache, nausea, lethargy, and a possible rash.

Some individuals carry *S. typhi* without presenting signs or symptoms (known as asymptomatic carriers) and continually shed them through their feces. These carriers often have the bacteria in the gallbladder or intestinal epithelium. Individuals consuming food or water contaminated with these feces can become infected.

S. typhi penetrate the intestinal mucosa, grow within the macrophages, and are transported through the body, most notably to the liver and gallbladder. Eventually, the macrophages lyse, releasing *S. typhi* into the bloodstream and lymphatic system. Mortality can result from ulceration and perforation of the intestine. A wide range of complications, such as pneumonia and jaundice, can occur with disseminated disease.

S. typhi have *Salmonella* pathogenicity islands (SPIs) that contain the genes for many of their virulence factors. Two examples of important typhoid toxins are the Vi antigen, which encodes for capsule production, and chimeric A2B5 toxin, which causes many of the signs and symptoms of the acute phase of typhoid fever.

Clinical examination and culture are used to make the diagnosis. The bacteria can be cultured from feces, urine, blood, or bone marrow. Serology, including ELISA, is used to identify the most pathogenic strains, but confirmation with DNA testing or culture is needed. A PCR test can also be used, but is not widely available.

The recommended antibiotic treatment involves fluoroquinolones, ceftriaxone, and azithromycin. Individuals must be extremely careful to avoid infecting others during treatment. Typhoid fever can be prevented through vaccination for individuals traveling to parts of the world where it is common.

Note:

- Why is serotyping particularly important in *Salmonella* infections and typhoid fever?

Note:

Typhoid Mary

Mary Mallon was an Irish immigrant who worked as a cook in New York in the early 20th century. Over seven years, from 1900 to 1907, Mallon worked for a number of different households, unknowingly spreading illness to the people who lived in each one. In 1906, one family hired George Soper, an expert in typhoid fever epidemics, to determine the cause of the illnesses in their household. Eventually, Soper tracked Mallon down and directly linked 22 cases of typhoid fever to her. He discovered that Mallon was a carrier for typhoid but was immune to it herself. Although active carriers had been recognized before, this was the first time that an asymptomatic carrier of infection had been identified.

Because she herself had never been ill, Mallon found it difficult to believe she could be the source of the illness. She fled from Soper and the authorities because she did not want to be quarantined or forced to give up her profession, which was relatively well paid for someone with her background. However, Mallon was eventually caught and kept in an isolation facility in the Bronx, where she remained until 1910, when the New York health department released her under the condition that she never again work with food. Unfortunately, Mallon did not comply, and she soon began working as a cook again. After new cases began to appear that resulted in the death of two individuals, the authorities tracked her down again and returned her to isolation, where she remained for 23 more years until her death in 1938. Epidemiologists were able to trace 51 cases of typhoid fever and three deaths directly to Mallon, who is unflatteringly remembered as “Typhoid Mary.”

The Typhoid Mary case has direct correlations in the health-care industry. Consider Kaci Hickox, an American nurse who treated Ebola patients in West Africa during the 2014 epidemic. After returning to the United States, Hickox was quarantined against her will for three days and later found not to have Ebola. Hickox vehemently opposed the quarantine. In an editorial published in the British newspaper *The Guardian*,[\[footnote\]](#) Hickox argued that quarantining asymptomatic health-care workers who had not tested positive for a disease would not only prevent such individuals from practicing their profession, but discourage others from volunteering to work in disease-ridden areas where health-care workers are desperately needed.

Kaci Hickox. “Stop Calling Me the ‘Ebola Nurse.’” *The Guardian*. November 17, 2014. <http://www.theguardian.com/commentisfree/2014/nov/17/stop-calling-me-ebola-nurse-kaci-hickox>. What is the responsibility of an individual like Mary Mallon to change her behavior to protect others? What happens when an individual believes that she is not a risk, but others believe that she is? How would you react if you were in Mallon’s shoes and were placed in a quarantine you did not believe was necessary, at the expense of your own freedom and possibly your career? Would it matter if you were definitely infected or not?

***E. coli* Infections**

The gram-negative rod *Escherichia coli* is a common member of the normal microbiota of the colon. Although the vast majority of *E. coli* strains are helpful commensal bacteria, some can be pathogenic and may cause dangerous diarrheal disease. The pathogenic strains have additional virulence factors such as type 1 fimbriae that promote colonization of the colon or may produce toxins (see [Virulence Factors of Bacterial and Viral Pathogens](#)). These virulence factors are acquired through horizontal gene transfer.

Extraintestinal disease can result if the bacteria spread from the gastrointestinal tract. Although these bacteria can be spread from person to person, they are often acquired through contaminated food or water. There are six recognized pathogenic groups of *E. coli*, but we will focus here on the four that are most commonly transmitted through food and water.

Enterotoxigenic *E. coli* (ETEC), also known as **traveler’s diarrhea**, causes diarrheal illness and is common in less developed countries. In Mexico, ETEC infection is called Montezuma’s Revenge. Following ingestion of contaminated food or water, infected individuals develop a watery diarrhea, abdominal cramps, **malaise** (a feeling of being unwell), and a low fever. ETEC produces a heat-stable enterotoxin similar to cholera toxin, and adhesins called colonization factors that help the bacteria to attach to the intestinal wall. Some strains of ETEC also produce heat-labile toxins. The disease is usually relatively mild and self-limiting. Diagnosis involves culturing and PCR. If needed, antibiotic treatment with fluoroquinolones, doxycycline, rifaximin, and trimethoprim-sulfamethoxazole (TMP/SMZ) may shorten infection duration. However, antibiotic resistance is a problem.

Enteroinvasive *E. coli* (EIEC) is very similar to shigellosis, including its pathogenesis of intracellular invasion into intestinal epithelial tissue. This bacterium carries a large plasmid that is involved in epithelial cell penetration. The illness is usually self-limiting, with symptoms including watery diarrhea, chills, cramps, malaise, fever, and dysentery. Culturing and PCR testing can be used for diagnosis. Antibiotic treatment is not recommended, so supportive therapy is used if needed.

Enteropathogenic *E. coli* (EPEC) can cause a potentially fatal diarrhea, especially in infants and those in less developed countries. Fever, vomiting, and diarrhea can lead to severe dehydration. These *E. coli* inject a protein (Tir) that attaches to the surface of the intestinal epithelial cells and triggers rearrangement of host cell actin from microvilli to pedestals. Tir also happens to be the receptor for Intimin, a surface protein produced by EPEC, thereby allowing *E. coli* to “sit” on the pedestal. The genes necessary for this pedestal formation are encoded on the locus for enterocyte effacement (LEE) pathogenicity island. As with ETEC, diagnosis involves culturing and PCR. Treatment is similar to that for ETEC.

The most dangerous strains are **enterohemorrhagic *E. coli* (EHEC)**, which are the strains capable of causing epidemics. In particular, the strain O157:H7 has been responsible for several recent outbreaks.

Recall that the O and H refer to surface antigens that contribute to pathogenicity and trigger a host immune response (“O” refers to the O-side chain of the lipopolysaccharide and the “H” refers to the flagella). Similar to EPEC, EHEC also forms pedestals. EHEC also produces a Shiga-like toxin. Because the genome of this bacterium has been sequenced, it is known that the Shiga toxin genes were most likely acquired through transduction (horizontal gene transfer). The Shiga toxin genes originated from *Shigella dysenteriae*. Prophage from a bacteriophage that previously infected *Shigella* integrated into the chromosome of *E. coli*. The Shiga-like toxin is often called verotoxin.

EHEC can cause disease ranging from relatively mild to life-threatening. Symptoms include bloody diarrhea with severe cramping, but no fever. Although it is often self-limiting, it can lead to hemorrhagic colitis and profuse bleeding. One possible complication is HUS. Diagnosis involves culture, often using MacConkey with sorbitol agar to differentiate between *E. coli* O157:H7, which does not ferment sorbitol, and other less virulent strains of *E. coli* that can ferment sorbitol.

Serological typing or PCR testing also can be used, as well as genetic testing for Shiga toxin. To distinguish EPEC from EHEC, because they both form pedestals on intestinal epithelial cells, it is necessary to test for genes encoding for both the Shiga-like toxin and for the LEE. Both EPEC and EHEC have LEE, but EPEC lacks the gene for Shiga toxin. Antibiotic therapy is not recommended and may worsen HUS because of the toxins released when the bacteria are killed, so supportive therapies must be used. [\[link\]](#) summarizes the characteristics of the four most common pathogenic groups.

Some Pathogenic Groups of <i>E. coli</i>				
Group	Virulence Factors and Genes	Signs and Symptoms	Diagnostic Tests	Treatment
Enterotoxigenic <i>E. coli</i> (ETEC)	Heat stable enterotoxin similar to cholera toxin	Relatively mild, watery diarrhea	Culturing, PCR	Self-limiting; if needed, fluoroquinolones, doxycycline, rifaximin, TMP/SMZ; antibiotic resistance is a problem

Some Pathogenic Groups of <i>E. coli</i>				
Group	Virulence Factors and Genes	Signs and Symptoms	Diagnostic Tests	Treatment
Enteroinvasive <i>E. coli</i> (EIEC)	<i>Inv</i> (invasive plasmid) genes	Relatively mild, watery diarrhea; dysentery or inflammatory colitis may occur	Culturing, PCR; testing for <i>inv</i> gene; additional assays to distinguish from <i>Shigella</i>	Supportive therapy only; antibiotics not recommended
Enteropathogenic <i>E. coli</i> (EPEC)	Locus of enterocyte effacement (LEE) pathogenicity island	Severe fever, vomiting, nonbloody diarrhea, dehydration; potentially fatal	Culturing, PCR; detection of LEE lacking Shiga-like toxin genes	Self-limiting; if needed, fluoroquinolones, doxycycline, rifaximin (TMP/SMZ); antibiotic resistance is a problem
Enterohemorrhagic <i>E. coli</i> (EHEC)	Verotoxin	May be mild or very severe; bloody diarrhea; may result in HUS	Culturing; plate on MacConkey agar with sorbitol agar as it does not ferment sorbitol; PCR detection of LEE containing Shiga-like toxin genes	Antibiotics are not recommended due to the risk of HUS

Note:

- Compare and contrast the virulence factors and signs and symptoms of infections with the four main *E. coli* groups.

Cholera and Other Vibrios

The gastrointestinal disease **cholera** is a serious infection often associated with poor sanitation, especially following natural disasters, because it is spread through contaminated water and food that has not been heated to temperatures high enough to kill the bacteria. It is caused by *Vibrio cholerae* serotype O1, a gram-negative, flagellated bacterium in the shape of a curved rod (vibrio). According to the CDC, cholera causes an estimated 3 to 5 million cases and 100,000 deaths each year.^[footnote] Centers for Disease Control and Prevention. *Cholera—Vibrio cholerae Infection*. Updated November 6, 2014. <http://www.cdc.gov/cholera/general>. Accessed Sept 14, 2016.

Because *V. cholerae* is killed by stomach acid, relatively large doses are needed for a few microbial cells to survive to reach the intestines and cause infection. The motile cells travel through the mucous layer of the intestines, where they attach to epithelial cells and release cholera enterotoxin. The toxin is an A-B toxin with activity through adenylate cyclase (see [Virulence Factors of Bacterial and Viral Pathogens](#)). Within the intestinal cell, cyclic AMP (cAMP) levels increase, which activates a chloride channel and results in the release of ions into the intestinal lumen. This increase in osmotic pressure in the lumen leads to water also entering the lumen. As the water and electrolytes leave the body, it causes rapid dehydration and electrolyte imbalance. Diarrhea is so profuse that it is often called “rice water stool,” and patients are placed on cots with a hole in them to monitor the fluid loss ([link](#)).

Cholera is diagnosed by taking a stool sample and culturing for *Vibrio*. The bacteria are oxidase positive and show non-lactose fermentation on MacConkey agar. Gram-negative lactose fermenters will produce red colonies while non-fermenters will produce white/colorless colonies. Gram-positive bacteria will not grow on MacConkey. Lactose fermentation is commonly used for pathogen identification because the normal microbiota generally ferments lactose while pathogens do not. *V. cholerae* may also be cultured on thiosulfate citrate bile salts sucrose (TCBS) agar, a selective and differential media for *Vibrio* spp., which produce a distinct yellow colony.

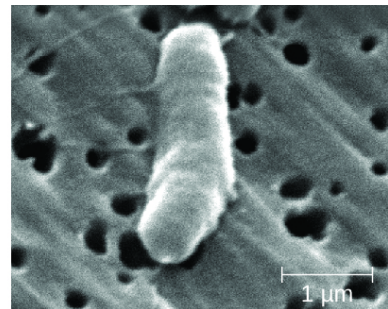
Cholera may be self-limiting and treatment involves rehydration and electrolyte replenishment. Although antibiotics are not typically needed, they can be used for severe or disseminated disease. Tetracyclines are recommended, but doxycycline, erythromycin, ofloxacin, ciprofloxacin, and TMP/SMZ may be used. Recent evidence suggests that azithromycin is also a good first-line antibiotic. Good sanitation—including appropriate sewage treatment, clean supplies for cooking, and purified drinking water—is important to prevent infection ([link](#))



(a)



(b)



(c)

(a) Outbreaks of cholera often occur in areas with poor sanitation or after natural disasters that compromise sanitation infrastructure. (b) At a cholera treatment center in Haiti, patients are

receiving intravenous fluids to combat the dehydrating effects of this disease. They often lie on a cot with a hole in it and a bucket underneath to allow for monitoring of fluid loss. (c) This scanning electron micrograph shows *Vibrio cholera*. (credit a, b: modification of work by Centers for Disease Control and Prevention; credit c: modification of work by Janice Carr, Centers for Disease Control and Prevention)

V. cholera is not the only *Vibrio* species that can cause disease. *V. parahaemolyticus* is associated with consumption of contaminated seafood and causes gastrointestinal illness with signs and symptoms such as watery diarrhea, nausea, fever, chills, and abdominal cramps. The bacteria produce a heat-stable hemolysin, leading to dysentery and possible disseminated disease. It also sometimes causes wound infections. *V. parahaemolyticus* is diagnosed using cultures from blood, stool, or a wound. As with *V. cholera*, selective medium (especially TCBS agar) works well. Tetracycline and ciprofloxacin can be used to treat severe cases, but antibiotics generally are not needed.

Vibrio vulnificus is found in warm seawater and, unlike *V. cholerae*, is not associated with poor sanitary conditions. The bacteria can be found in raw seafood, and ingestion causes gastrointestinal illness. It can also be acquired by individuals with open skin wounds who are exposed to water with high concentrations of the pathogen. In some cases, the infection spreads to the bloodstream and causes septicemia. Skin infection can lead to edema, ecchymosis (discoloration of skin due to bleeding), and abscesses. Patients with underlying disease have a high fatality rate of about 50%. It is of particular concern for individuals with chronic liver disease or who are otherwise immunodeficient because a healthy immune system can often prevent infection from developing. *V. vulnificus* is diagnosed by culturing for the pathogen from stool samples, blood samples, or skin abscesses. Adult patients are treated with doxycycline combined with a third generation cephalosporin or with fluoroquinolones, and children are treated with TMP/SMZ.

Two other vibrios, *Aeromonas hydrophila* and *Plesiomonas shigelloides*, are also associated with marine environments and raw seafood; they can also cause gastroenteritis. Like *V. vulnificus*, *A. hydrophila* is more often associated with infections in wounds, generally those acquired in water. In some cases, it can also cause septicemia. Other species of *Aeromonas* can cause illness. *P. shigelloides* is sometimes associated with more serious systemic infections if ingested in contaminated food or water. Culture can be used to diagnose *A. hydrophila* and *P. shigelloides* infections, for which antibiotic therapy is generally not needed. When necessary, tetracycline and ciprofloxacin, among other antibiotics, may be used for treatment of *A. hydrophila*, and fluoroquinolones and trimethoprim are the effective treatments for *P. shigelloides*.

Note:

- How does *V. cholera* infection cause rapid dehydration?

***Campylobacter jejuni* Gastroenteritis**

Campylobacter is a genus of gram-negative, spiral or curved bacteria. They may have one or two flagella. *Campylobacter jejuni* gastroenteritis, a form of campylobacteriosis, is a widespread illness

that is caused by *Campylobacter jejuni*. The primary route of transmission is through poultry that becomes contaminated during slaughter. Handling of the raw chicken in turn contaminates cooking surfaces, utensils, and other foods. Unpasteurized milk or contaminated water are also potential vehicles of transmission. In most cases, the illness is self-limiting and includes fever, diarrhea, cramps, vomiting, and sometimes dysentery. More serious signs and symptoms, such as bacteremia, meningitis, pancreatitis, cholecystitis, and hepatitis, sometimes occur. It has also been associated with autoimmune conditions such as Guillain-Barré syndrome, a neurological disease that occurs after some infections and results in temporary paralysis. HUS following infection can also occur. The virulence in many strains is the result of hemolysin production and the presence of *Campylobacter* cytolethal distending toxin (CDT), a powerful deoxyribonuclease (DNase) that irreversibly damages host cell DNA.

Diagnosis involves culture under special conditions, such as elevated temperature, low oxygen tension, and often medium supplemented with antimicrobial agents. These bacteria should be cultured on selective medium (such as Campy CV, charcoal selective medium, or cefaperazone charcoal deoxycholate agar) and incubated under microaerophilic conditions for at least 72 hours at 42 °C. Antibiotic treatment is not usually needed, but erythromycin or ciprofloxacin may be used.

Peptic Ulcers

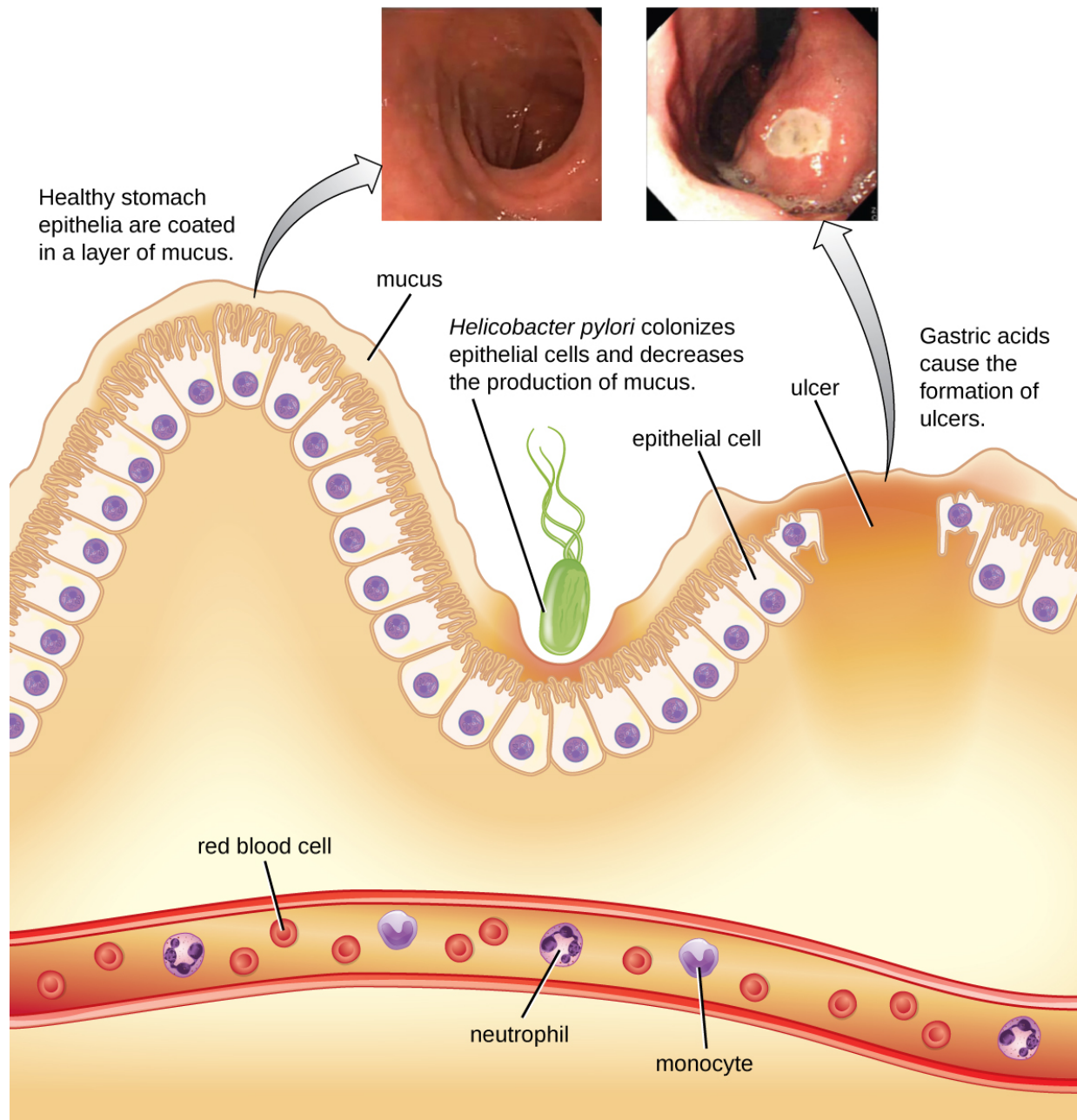
The gram-negative bacterium *Helicobacter pylori* is able to tolerate the acidic environment of the human stomach and has been shown to be a major cause of **peptic ulcers**, which are ulcers of the stomach or duodenum. The bacterium is also associated with increased risk of stomach cancer ([[link](#)]). According to the CDC, approximately two-thirds of the population is infected with *H. pylori*, but less than 20% have a risk of developing ulcers or stomach cancer. *H. pylori* is found in approximately 80% of stomach ulcers and in over 90% of duodenal ulcers.[[footnote](#)]

Centers for Disease Control and Prevention. “*Helicobacter pylori*: Fact Sheet for Health Care Providers.” Updated July 1998. <http://www.cdc.gov/ulcer/files/hpfacts.pdf>.

H. pylori colonizes epithelial cells in the stomach using pili for adhesion. These bacteria produce urease, which stimulates an immune response and creates ammonia that neutralizes stomach acids to provide a more hospitable microenvironment. The infection damages the cells of the stomach lining, including those that normally produce the protective mucus that serves as a barrier between the tissue and stomach acid. As a result, inflammation (gastritis) occurs and ulcers may slowly develop. Ulcer formation can also be caused by toxin activity. It has been reported that 50% of clinical isolates of *H. pylori* have detectable levels of exotoxin activity *in vitro*. [[footnote](#)] This toxin, VacA, induces vacuole formation in host cells. VacA has no primary sequence homology with other bacterial toxins, and in a mouse model, there is a correlation between the presence of the toxin gene, the activity of the toxin, and gastric epithelial tissue damage.

T. L. Cover. “The Vacuolating Cytotoxin of *Helicobacter pylori*.” *Molecular Microbiology* 20 (1996) 2: pp. 241–246. <http://www.ncbi.nlm.nih.gov/pubmed/8733223>.

Signs and symptoms include nausea, lack of appetite, bloating, burping, and weight loss. Bleeding ulcers may produce dark stools. If no treatment is provided, the ulcers can become deeper, more tissues can be involved, and stomach perforation can occur. Because perforation allows digestive enzymes and acid to leak into the body, it is a very serious condition.



Helicobacter infection decreases mucus production and causes peptic ulcers. (credit top left photo: modification of work by "Santhosh Thomas"/YouTube; credit top right photo: modification of work by Moriya M, Uehara A, Okumura T, Miyamoto M, and Kohgo Y)

To diagnose *H. pylori* infection, multiple methods are available. In a breath test, the patient swallows radiolabeled urea. If *H. pylori* is present, the bacteria will produce urease to break down the urea. This reaction produces radiolabeled carbon dioxide that can be detected in the patient's breath. Blood testing can also be used to detect antibodies to *H. pylori*. The bacteria themselves can be detected using either a stool test or a stomach wall biopsy.

Antibiotics can be used to treat the infection. However, unique to *H. pylori*, the recommendation from the US Food and Drug Administration is to use a triple therapy. The current protocols are 10 days of treatment with omeprazole, amoxicillin, and clarithromycin (OAC); 14 days of treatment with bismuth subsalicylate, metronidazole, and tetracycline (BMT); or 10 or 14 days of treatment with lansoprazole, amoxicillin, and clarithromycin (LAC). Omeprazole, bismuth subsalicylate, and lansoprazole are not antibiotics but are instead used to decrease acid levels because *H. pylori* prefers acidic environments.

Although treatment is often valuable, there are also risks to *H. pylori* eradication. Infection with *H. pylori* may actually protect against some cancers, such as esophageal adenocarcinoma and gastroesophageal reflux disease. [\[footnote\]](#)[\[footnote\]](#)

Martin J. Blaser. “Disappearing Microbiota: *Helicobacter pylori* Protection against Esophageal Adenocarcinoma.” *Cancer Prevention Research* 1 (2008) 5: pp. 308–311.

<http://cancerpreventionresearch.aacrjournals.org/content/1/5/308.full.pdf+html>.

Ivan F. N. Hung and Benjamin C. Y. Wong. “Assessing the Risks and Benefits of Treating *Helicobacter pylori* Infection.” *Therapeutic Advances in Gastroenterology* 2 (2009) 3: pp, 141–147. doi: 10.1177/1756283X08100279.

Note:

- How does *H. pylori* cause peptic ulcers?

***Clostridium perfringens* Gastroenteritis**

***Clostridium perfringens* gastroenteritis** is a generally mild foodborne disease that is associated with undercooked meats and other foods. *C. perfringens* is a gram-positive, rod-shaped, endospore-forming anaerobic bacterium that is tolerant of high and low temperatures. At high temperatures, the bacteria can form endospores that will germinate rapidly in foods or within the intestine. Food poisoning by type A strains is common. This strain always produces an enterotoxin, sometimes also present in other strains, that causes the clinical symptoms of cramps and diarrhea. A more severe form of the illness, called pig-bel or enteritis necroticans, causes hemorrhaging, pain, vomiting, and bloating. Gangrene of the intestines may result. This form has a high mortality rate but is rare in the United States.

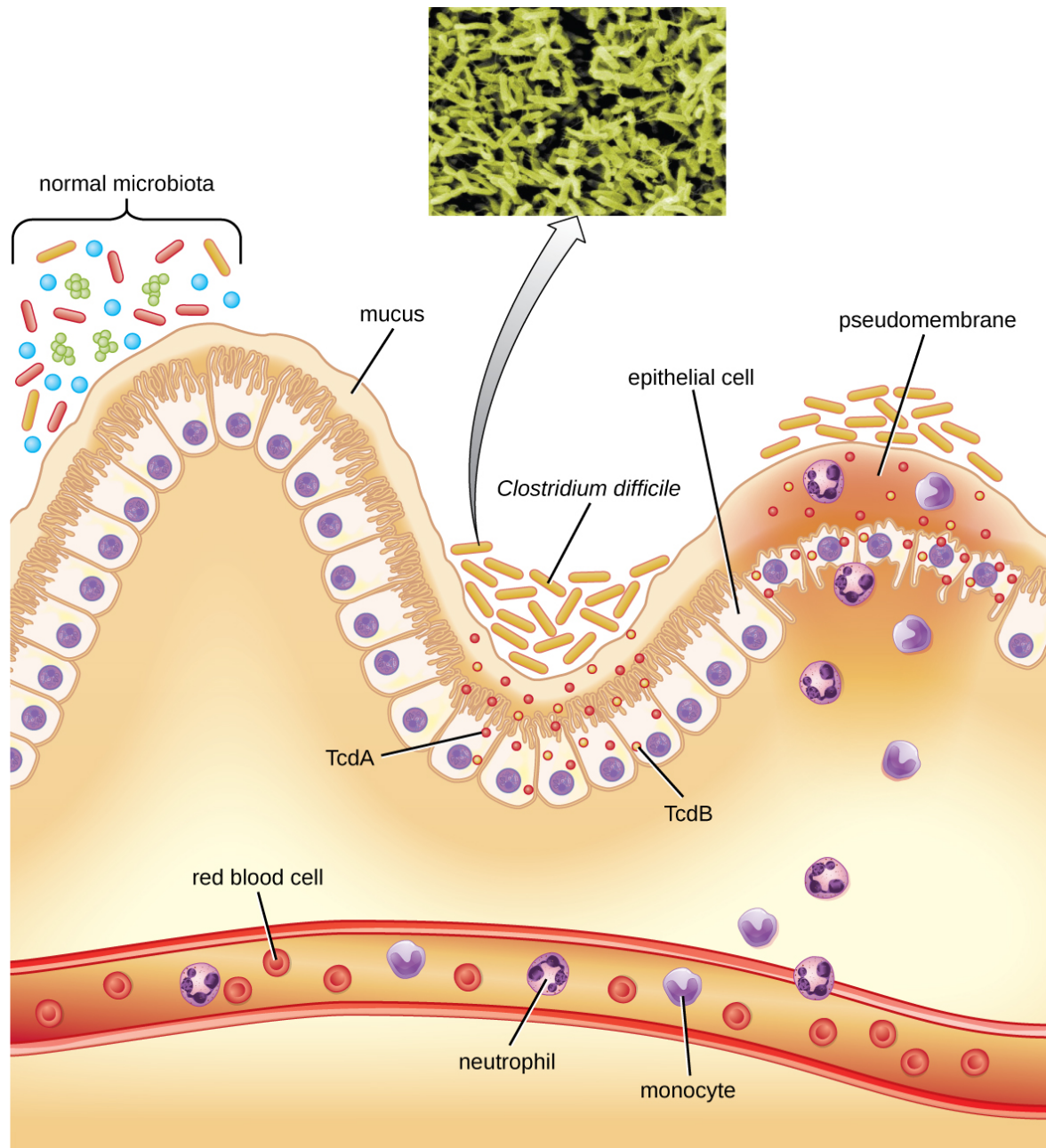
Diagnosis involves detecting the *C. perfringens* toxin in stool samples using either molecular biology techniques (PCR detection of the toxin gene) or immunology techniques (ELISA). The bacteria itself may also be detected in foods or in fecal samples. Treatment includes rehydration therapy, electrolyte replacement, and intravenous fluids. Antibiotics are not recommended because they can damage the balance of the microbiota in the gut, and there are concerns about antibiotic resistance. The illness can be prevented through proper handling and cooking of foods, including prompt refrigeration at sufficiently low temperatures and cooking food to a sufficiently high temperature.

Clostridium difficile

Clostridium difficile is a gram-positive rod that can be a commensal bacterium as part of the normal microbiota of healthy individuals. When the normal microbiota is disrupted by long-term antibiotic use, it can allow the overgrowth of this bacterium, resulting in **antibiotic-associated diarrhea** caused by *C. difficile*. Antibiotic-associated diarrhea can also be considered a nosocomial disease. Patients at

the greatest risk of *C. difficile* infection are those who are immunocompromised, have been in health-care settings for extended periods, are older, have recently taken antibiotics, have had gastrointestinal procedures done, or use proton pump inhibitors, which reduce stomach acidity and allow proliferation of *C. difficile*. Because this species can form endospores, it can survive for extended periods of time in the environment under harsh conditions and is a considerable concern in health-care settings.

This bacterium produces two toxins, *Clostridium difficile* toxin A (TcdA) and *Clostridium difficile* toxin B (TcdB). These toxins inactivate small GTP-binding proteins, resulting in actin condensation and cell rounding, followed by cell death. Infections begin with focal necrosis, then ulceration with exudate, and can progress to **pseudomembranous colitis**, which involves inflammation of the colon and the development of a pseudomembrane of fibrin containing dead epithelial cells and leukocytes ([link](#)). Watery diarrhea, dehydration, fever, loss of appetite, and abdominal pain can result. Perforation of the colon can occur, leading to septicemia, shock, and death. *C. difficile* is also associated with necrotizing enterocolitis in premature babies and neutropenic enterocolitis associated with cancer therapies.



Clostridium difficile is able to colonize the mucous membrane of the colon when the normal microbiota is disrupted. The toxins TcdA and TcdB trigger an immune response, with neutrophils and monocytes migrating from the bloodstream to the site of infection. Over time, inflammation and dead cells contribute to the development of a pseudomembrane. (credit micrograph: modification of work by Janice Carr, Centers for Disease Control and Prevention)

Diagnosis is made by considering the patient history (such as exposure to antibiotics), clinical presentation, imaging, endoscopy, lab tests, and other available data. Detecting the toxin in stool

samples is used to confirm diagnosis. Although culture is preferred, it is rarely practical in clinical practice because the bacterium is an obligate anaerobe. Nucleic acid amplification tests, including PCR, are considered preferable to ELISA testing for molecular analysis.

The first step of conventional treatment is to stop antibiotic use, and then to provide supportive therapy with electrolyte replacement and fluids. Metronidazole is the preferred treatment if the *C. difficile* diagnosis has been confirmed. Vancomycin can also be used, but it should be reserved for patients for whom metronidazole was ineffective or who meet other criteria (e.g., under 10 years of age, pregnant, or allergic to metronidazole).

A newer approach to treatment, known as a fecal transplant, focuses on restoring the microbiota of the gut in order to combat the infection. In this procedure, a healthy individual donates a stool sample, which is mixed with saline and transplanted to the recipient via colonoscopy, endoscopy, sigmoidoscopy, or enema. It has been reported that this procedure has greater than 90% success in resolving *C. difficile* infections.[\[footnote\]](#)

Faith Rohlke and Neil Stollman. “Fecal Microbiota Transplantation in Relapsing *Clostridium difficile* Infection,” *Therapeutic Advances in Gastroenterology* 5 (2012) 6: 403–420. doi: 10.1177/1756283X12453637.

Note:

- How does antibiotic use lead to *C. difficile* infections?

Foodborne Illness Due to *Bacillus cereus*

Bacillus cereus, commonly found in soil, is a gram-positive endospore-forming bacterium that can sometimes cause foodborne illness. *B. cereus* endospores can survive cooking and produce enterotoxins in food after it has been heated; illnesses often occur after eating rice and other prepared foods left at room temperature for too long. The signs and symptoms appear within a few hours of ingestion and include nausea, pain, and abdominal cramps. *B. cereus* produces two toxins: one causing diarrhea, and the other causing vomiting. More severe signs and symptoms can sometimes develop.

Diagnosis can be accomplished by isolating bacteria from stool samples or vomitus and uneaten infected food. Treatment involves rehydration and supportive therapy. Antibiotics are not typically needed, as the illness is usually relatively mild and is due to toxin activity.

Foodborne Illness Due to *Yersinia*

The genus *Yersinia* is best known for *Yersinia pestis*, a gram-negative rod that causes the plague. However, *Y. enterocolitica* and *Y. pseudotuberculosis* can cause gastroenteritis. The infection is generally transmitted through the fecal-oral route, with ingestion of food or water that has been contaminated by feces. Intoxication can also result because of the activity of its endotoxin and exotoxins (enterotoxin and cytotoxin necrotizing factor). The illness is normally relatively mild and self-limiting. However, severe diarrhea and dysentery can develop in infants. In adults, the infection can spread and cause complications such as reactive arthritis, thyroid disorders, endocarditis,

glomerulonephritis, eye inflammation, and/or erythema nodosum. Bacteremia may develop in rare cases.

Diagnosis is generally made by detecting the bacteria in stool samples. Samples may also be obtained from other tissues or body fluids. Treatment is usually supportive, including rehydration, without antibiotics. If bacteremia or other systemic disease is present, then antibiotics such as fluoroquinolones, aminoglycosides, doxycycline, and trimethoprim-sulfamethoxazole may be used. Recovery can take up to two weeks.

Note:

- Compare and contrast foodborne illnesses due to *B. cereus* and *Yersinia*.

Note:

Bacterial Infections of the Gastrointestinal Tract

Bacterial infections of the gastrointestinal tract generally occur when bacteria or bacterial toxins are ingested in contaminated food or water. Toxins and other virulence factors can produce gastrointestinal inflammation and general symptoms such as diarrhea and vomiting. Bacterial GI infections can vary widely in terms of severity and treatment. Some can be treated with antibiotics, but in other cases antibiotics may be ineffective in combating toxins or even counterproductive if they compromise the GI microbiota. [\[link\]](#) and [\[link\]](#) the key features of common bacterial GI infections.

Bacterial Infections of the GI Tract					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
<i>Bacillus cereus</i> infection	<i>Bacillus cereus</i>	Nausea, pain, abdominal cramps, diarrhea, or vomiting	Ingestion of contaminated rice or meat, even after cooking	Testing stool sample, vomitus, or uneaten food for presence of bacteria	None
<i>Campylobacter jejuni</i> gastroenteritis	<i>Campylobacter jejuni</i>	Fever, diarrhea, cramps, vomiting, and sometimes dysentery; sometimes more severe organ or autoimmune effects	Ingestion of unpasteurized milk, under-cooked chicken, or contaminated water	Culture on selective medium with elevated temperature and low oxygen concentration	Generally none; erythromycin or ciprofloxacin if necessary
Cholera	<i>Vibrio cholerae</i>	Severe diarrhea and fluid loss, potentially leading to shock, renal failure, and death	Ingestion of contaminated water or food	Culture on selective medium (TCBS agar); distinguished as oxidase positive with fermentative metabolisms	Generally none; tetracyclines, azithromycin, others if necessary
<i>Clostridium difficile</i> infection	<i>Clostridium difficile</i>	Pseudomembranous colitis, watery diarrhea, fever, abdominal pain, loss of appetite, dehydration; in severe cases, perforation of the colon, septicemia, shock, and death	Overgrowth of <i>C. difficile</i> in the normal microbiota due to antibiotic use; hospital-acquired infections in immunocompromised patients	Detection of toxin in stool, nucleic acid amplification tests (e.g., PCR)	Discontinuation of previous antibiotic treatment; metronidazole or vancomycin
<i>Clostridium perfringens</i> gastroenteritis	<i>Clostridium perfringens</i> (especially type A)	Mild cramps and diarrhea in most cases; in rare cases, hemorrhaging, vomiting, intestinal gangrene, and death	Ingestion of undercooked meats containing <i>C. perfringens</i> endospores	Detection of toxin or bacteria in stool or uneaten food	None
<i>E. coli</i> infection	ETEC, EPEC, EIEC, EHEC	Watery diarrhea, dysentery, cramps, malaise, fever, chills, dehydration; in EHEC, possible severe complications such as hemolytic uremic syndrome	Ingestion of contaminated food or water	Tissue culture, immunochemical assays, PCR, gene probes	Not recommended for EIEC and EHEC; fluoroquinolones, doxycycline, rifaximin, and TMP/SMZ possible for ETEC and EPEC

Bacterial Infections of the GI Tract (continued)					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Peptic ulcers	<i>Helicobacter pylori</i>	Nausea, bloating, burping, lack of appetite, weight loss, perforation of stomach, blood in stools	Normal flora, can also be acquired via saliva; fecal-oral route via contaminated food and water	Breath test, detection of antibodies in blood, detection of bacteria in stool sample or stomach biopsy	Amoxicillin, clarithromycin, metronidazole, tetracycline, lansoprazole; antacids may also be given in combination with antibiotics
Salmonellosis	<i>Salmonella enterica</i> , serotype Enteritidis	Fever, nausea, vomiting, abdominal cramps, headache, diarrhea; can be fatal in infants	Ingestion of contaminated food, handling of eggshells or contaminated animals	Culturing, serotyping and DNA fingerprinting	Not generally recommended; fluoroquinolones, ampicillin, others for immunocompromised patients
Shigella dysentery	<i>Shigella dysenteriae</i> , <i>S. flexneri</i> , <i>S. boydii</i> , and <i>S. sonnei</i>	Abdominal cramps, fever, diarrhea, dysentery; possible complications: reactive arthritis and hemolytic uremic syndrome	Fecal-oral route via contaminated food and water	Testing of stool samples for presence of blood and leukocytes; culturing, PCR, immunoassay for <i>S. dysenteriae</i>	Ciprofloxacin, azithromycin
Staphylococcal food poisoning	<i>Staphylococcus aureus</i>	Rapid-onset nausea, diarrhea, vomiting lasting 24–48 hours; possible dehydration and change in blood pressure and heart rate	Ingestion of raw or undercooked meat or dairy products contaminated with staphylococcal enterotoxins	ELISA to detect enterotoxins in uneaten food, stool, or vomitus	None
Typhoid fever	<i>S. enterica</i> , subtypes Typhi or Paratyphi	Aches, headaches, nausea, lethargy, diarrhea or constipation, possible rash; lethal perforation of intestine can occur	Fecal-oral route; may be spread by asymptomatic carriers	Culture of blood, stool, or bone marrow, serologic tests; PCR tests when available	Fluoroquinolones, ceftriaxone, azithromycin; preventive vaccine available
Yersinia infection	<i>Yersinia enterocolitica</i> , <i>Y. pseudotuberculosis</i>	Generally mild diarrhea and abdominal cramps; in some cases, bacteremia can occur, leading to severe complications	Fecal-oral route, typically via contaminated food or water	Testing stool samples, tissues, body fluids	Generally none; fluoroquinolones, aminoglycosides, others for systemic infections

Note:

Part 2

At the hospital, Carli's doctor began to think about possible causes of her severe gastrointestinal distress. One possibility was food poisoning, but no one else in her family was sick. The doctor asked about what Carli had eaten the previous day; her mother mentioned that she'd had eggs for lunch, and that they may have been a little undercooked. The doctor took a sample of Carli's stool and sent it for laboratory testing as part of her workup. She suspected that Carli could have a case of bacterial or viral gastroenteritis, but she needed to know the cause in order to prescribe an appropriate treatment.

In the laboratory, technicians microscopically identified gram-negative bacilli in Carli's stool sample. They also established a pure culture of the bacteria and analyzed it for antigens. This testing showed that the causative agent was *Salmonella*.

- What should the doctor do now to treat Carli?

Jump to the [next](#) Clinical Focus box. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Major causes of gastrointestinal illness include *Salmonella* spp., *Staphylococcus* spp., *Helicobacter pylori*, *Clostridium perfringens*, *Clostridium difficile*, *Bacillus cereus*, and *Yersinia* bacteria.
- *C. difficile* is an important cause of hospital acquired infection.
- *Vibrio cholerae* causes **cholera**, which can be a severe diarrheal illness.
- Different strains of *E. coli*, including **ETEC**, **EPEC**, **EIEC**, and **EHEC**, cause different illnesses with varying degrees of severity.
- *H. pylori* is associated with **peptic ulcers**.
- *Salmonella enterica* serotypes can cause **typhoid fever**, a more severe illness than **salmonellosis**.
- Rehydration and other supportive therapies are often used as general treatments.
- Careful antibiotic use is required to reduce the risk of causing *C. difficile* infections and when treating antibiotic-resistant infections.

Multiple Choice

Exercise:

Problem:

Which type of *E. coli* infection can be severe with life-threatening consequences such as hemolytic uremic syndrome?

- a. ETEC
- b. EPEC
- c. EHEC
- d. EIEC

Solution:

C

Exercise:

Problem: Which species of *Shigella* has a type that produces Shiga toxin?

- a. *S. boydii*
- b. *S. flexneri*
- c. *S. dysenteriae*
- d. *S. sonnei*

Solution:

C

Exercise:

Problem: Which type of bacterium produces an A-B toxin?

- a. *Salmonella*
- b. *Vibrio cholera*
- c. ETEC
- d. *Shigella dysenteriae*

Solution:

B

Fill in the Blank

Exercise:

Problem: Antibiotic associated pseudomembranous colitis is caused by _____.

Solution:

Clostridium difficile

Critical Thinking

Exercise:

Problem:

Why does use of antibiotics and/or proton pump inhibitors contribute to the development of *C. difficile* infections?

Exercise:

Problem: Why did scientists initially think it was unlikely that a bacterium caused peptic ulcers?

Exercise:

Problem:

Does it make a difference in treatment to know if a particular illness is caused by a bacterium (an infection) or a toxin (an intoxication)?

Viral Infections of the Gastrointestinal Tract

LEARNING OBJECTIVES

- Identify the most common viruses that can cause infections of the GI tract
- Compare the major characteristics of specific viral diseases affecting the GI tract and liver

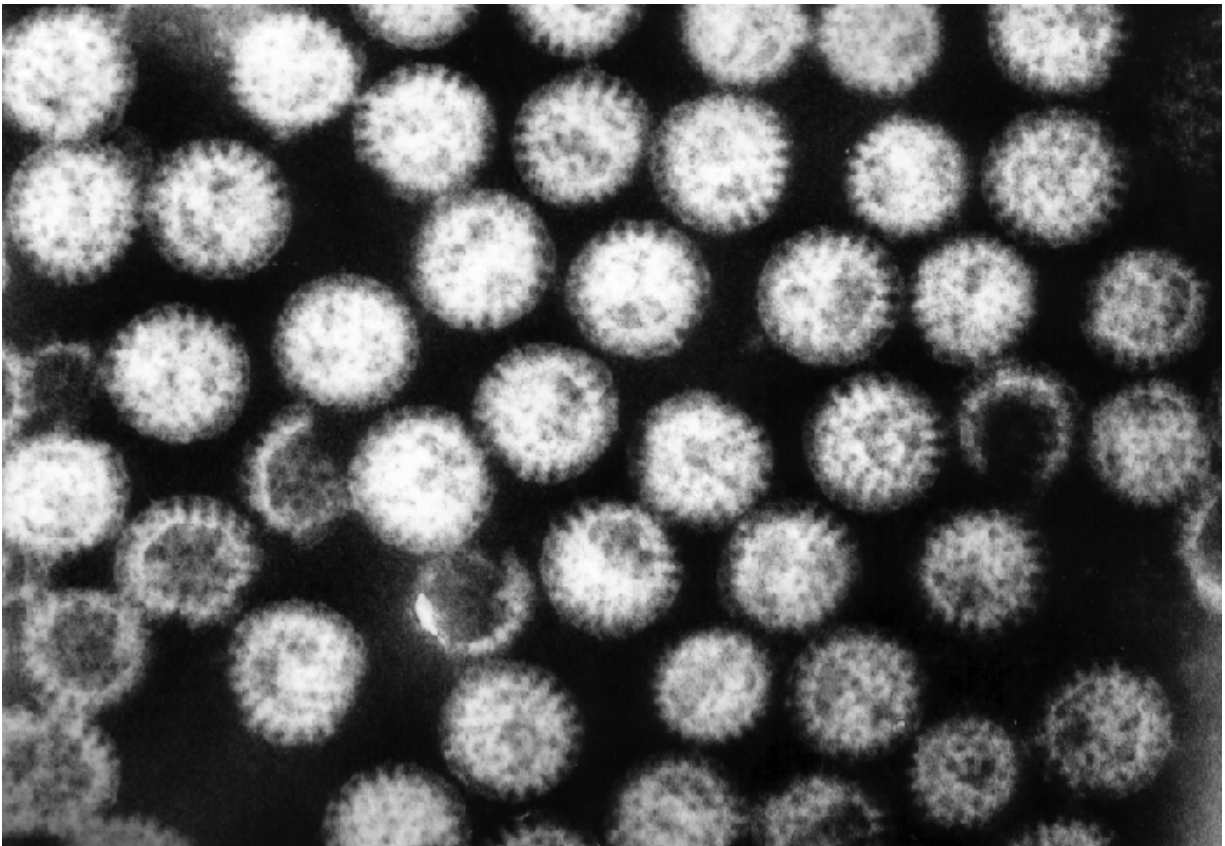
In the developing world, acute viral gastroenteritis is devastating and a leading cause of death for children.[\[footnote\]](#) Worldwide, diarrhea is the second leading cause of mortality for children under age five, and 70% of childhood gastroenteritis is viral.[\[footnote\]](#) As discussed, there are a number of bacteria responsible for diarrhea, but viruses can also cause diarrhea. *E. coli* and rotavirus are the most common causative agents in the developing world. In this section, we will discuss rotaviruses and other, less common viruses that can also cause gastrointestinal illnesses.

Caleb K. King, Roger Glass, Joseph S. Bresee, Christopher Duggan. “Managing Acute Gastroenteritis Among Children: Oral Rehydration, Maintenance, and Nutritional Therapy.” *MMWR* 52 (2003) RR16: pp. 1–16. <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5216a1.htm>.

Elizabeth Jane Elliott. “Acute Gastroenteritis in Children.” *British Medical Journal* 334 (2007) 7583: 35–40, doi: 10.1136/bmj.39036.406169.80; S. Ramani and G. Kang. “Viruses Causing Diarrhoea in the Developing World.” *Current Opinions in Infectious Diseases* 22 (2009) 5: pp. 477–482. doi: 10.1097/QCO.0b013e328330662f; Michael Vincent F Tablang. “Viral Gastroenteritis.” *Medscape*. <http://emedicine.medscape.com/article/176515-overview>.

Gastroenteritis Caused by Rotaviruses

Rotaviruses are double-stranded RNA viruses in the family Reoviridae. They are responsible for common diarrheal illness, although prevention through vaccination is becoming more common. The virus is primarily spread by the fecal-oral route ([link](#)).



Rotaviruses in a fecal sample are visualized using electron microscopy.
(credit: Dr. Graham Beards)

These viruses are widespread in children, especially in day-care centers. The CDC estimates that 95% of children in the United States have had at least one rotavirus infection by the time they reach age five.[\[footnote\]](#) Due to the memory of the body's immune system, adults who come into contact

with rotavirus will not contract the infection or, if they do, are asymptomatic. The elderly, however, are vulnerable to rotavirus infection due to weakening of the immune system with age, so infections can spread through nursing homes and similar facilities. In these cases, the infection may be transmitted from a family member who may have subclinical or clinical disease. The virus can also be transmitted from contaminated surfaces, on which it can survive for some time.

Centers for Disease Control and Prevention. "Rotavirus," *The Pink Book*. Updated September 8, 2015.

<http://www.cdc.gov/vaccines/pubs/pinkbook/rota.html>.

Infected individuals exhibit fever, vomiting, and diarrhea. The virus can survive in the stomach following a meal, but is normally found in the small intestines, particularly the epithelial cells on the villi. Infection can cause food intolerance, especially with respect to lactose. The illness generally appears after an incubation period of about two days and lasts for approximately one week (three to eight days). Without supportive treatment, the illness can cause severe fluid loss, dehydration, and even death. Even with milder illness, repeated infections can potentially lead to malnutrition, especially in developing countries, where rotavirus infection is common due to poor sanitation and lack of access to clean drinking water. Patients (especially children) who are malnourished after an episode of diarrhea are more susceptible to future diarrheal illness, increasing their risk of death from rotavirus infection.

The most common clinical tool for diagnosis is enzyme immunoassay, which detects the virus from fecal samples. Latex agglutination assays are also used. Additionally, the virus can be detected using electron microscopy and RT-PCR.

Treatment is supportive with oral rehydration therapy. Preventive vaccination is also available. In the United States, rotavirus vaccines are part of the standard vaccine schedule and administration follows the guidelines of the World Health Organization (WHO). The WHO recommends that all infants worldwide receive the rotavirus vaccine, the first dose between six and 15 weeks of age and the second before 32 weeks.

[\[footnote\]](#)

World Health Organization. "Rotavirus." *Immunization, Vaccines, and Biologicals*. Updated April 21, 2010.
<http://www.who.int/immunization/topics/rotavirus/en/>.

Gastroenteritis Caused by Noroviruses

Noroviruses, commonly identified as Norwalk viruses, are caliciviruses. Several strains can cause gastroenteritis. There are millions of cases a year, predominately in infants, young children, and the elderly. These viruses are easily transmitted and highly contagious. They are known for causing widespread infections in groups of people in confined spaces, such as on cruise ships. The viruses can be transmitted through direct contact, through touching contaminated surfaces, and through contaminated food. Because the virus is not killed by disinfectants used at standard concentrations for killing bacteria, the risk of transmission remains high, even after cleaning.

The signs and symptoms of norovirus infection are similar to those for rotavirus, with watery diarrhea, mild cramps, and fever. Additionally, these viruses sometimes cause projectile vomiting. The illness is usually relatively mild, develops 12 to 48 hours after exposure, and clears within a couple of days without treatment. However, dehydration may occur.

Norovirus can be detected using PCR or enzyme immunoassay (EIA) testing. RT-qPCR is the preferred approach as EIA is insufficiently sensitive. If EIA is used for rapid testing, diagnosis should be confirmed using PCR. No medications are available, but the illness is usually self-limiting. Rehydration therapy and electrolyte replacement may be used. Good hygiene, hand washing, and careful food preparation reduce the risk of infection.

Gastroenteritis Caused by Astroviruses

Astroviruses are single-stranded RNA viruses (family Astroviridae) that can cause severe gastroenteritis, especially in infants and children. Signs and symptoms include diarrhea, nausea, vomiting, fever, abdominal pain, headache, and malaise. The viruses are transmitted through the fecal-oral route (contaminated food or water). For diagnosis, stool samples are

analyzed. Testing may involve enzyme immunoassays and immune electron microscopy. Treatment involves supportive rehydration and electrolyte replacement if needed.

Note:

- Why are rotaviruses, noroviruses, and astroviruses more common in children?

Note:

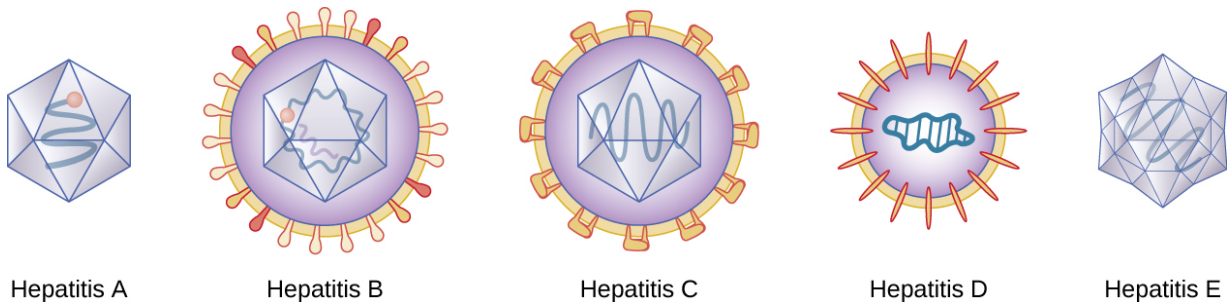
Viral Infections of the Gastrointestinal Tract

A number of viruses can cause gastroenteritis, characterized by inflammation of the GI tract and other signs and symptoms with a range of severities. As with bacterial GI infections, some cases can be relatively mild and self-limiting, while others can become serious and require intensive treatment. Antimicrobial drugs are generally not used to treat viral gastroenteritis; generally, these illnesses can be treated effectively with rehydration therapy to replace fluids lost in bouts of diarrhea and vomiting. Because most viral causes of gastroenteritis are quite contagious, the best preventive measures involve avoiding and/or isolating infected individuals and limiting transmission through good hygiene and sanitation.

Viral Causes of Gastroenteritis					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Vaccine
Astrovirus gastroenteritis	Astroviruses	Fever, headache, abdominal pain, malaise, diarrhea, vomiting	Fecal-oral route, contaminated food or water	Enzyme immunoassays, immune electron microscopy	None
Norovirus gastroenteritis	Noroviruses	Fever, diarrhea, projectile vomiting, dehydration; generally self-limiting within two days	Highly contagious via direct contact or contact with contaminated food or fomites	Rapid enzyme immunoassay confirmed with RT-qPCR	None
Rotavirus gastroenteritis	Rotaviruses	Fever, diarrhea, vomiting, severe dehydration; recurring infections can lead to malnutrition and death	Fecal-oral route; children and elderly most susceptible	Enzyme immunoassay of stool sample, latex agglutination assays, RT-PCR	Preventive vaccine recommended for infants

Hepatitis

Hepatitis is a general term meaning inflammation of the liver, which can have a variety of causes. In some cases, the cause is viral infection. There are five main hepatitis viruses that are clinically significant: hepatitisviruses A (HAV), B (HBV), C (HCV), D, (HDV) and E (HEV) ([\[link\]](#)). Note that other viruses, such as Epstein-Barr virus (EBV), yellow fever, and cytomegalovirus (CMV) can also cause hepatitis and are discussed in [Viral Infections of the Circulatory and Lymphatic Systems](#).

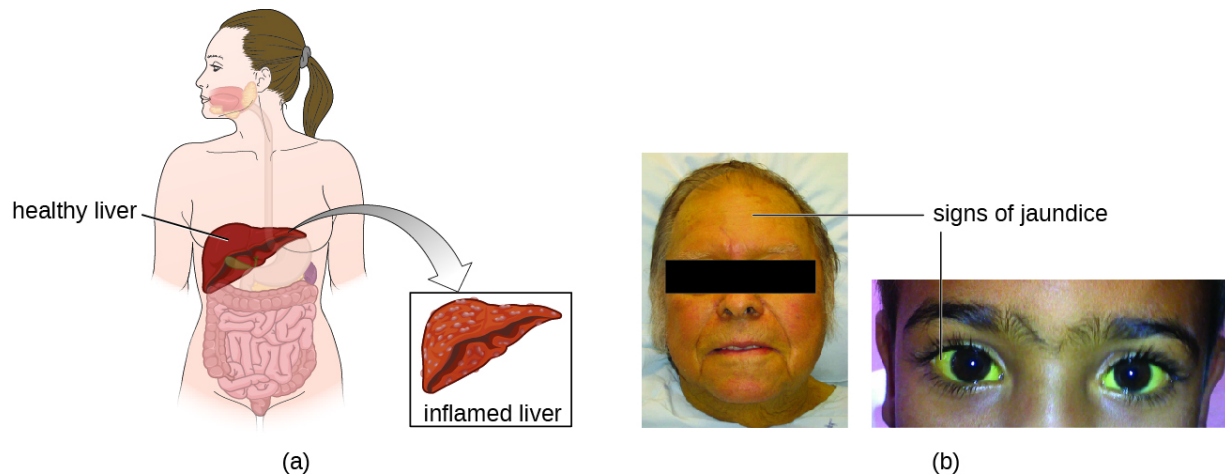


Five main types of viruses cause hepatitis. HAV is a non-enveloped

ssRNA(+) virus and is a member of the picornavirus family (Baltimore Group IV). HBV is a dsDNA enveloped virus, replicates using reverse transcriptase, and is a member of the hepadnavirus family (Baltimore Group VII). HCV is an enveloped ssRNA(+) virus and is a member of the flavivirus family (Baltimore Group IV). HDV is an enveloped ssRNA(−) that is circular (Baltimore Group V). This virus can only propagate in the presence of HBV. HEV is a non-enveloped ssRNA(+) virus and a member of the hepeviridae family (Baltimore Group IV).

Although the five hepatitis viruses differ, they can cause some similar signs and symptoms because they all have an affinity for hepatocytes (liver cells). HAV and HEV can be contracted through ingestion while HBV, HCV, and HDV are transmitted by parenteral contact. It is possible for individuals to become long term or chronic carriers of hepatitis viruses.

The virus enters the blood (viremia), spreading to the spleen, the kidneys, and the liver. During viral replication, the virus infects hepatocytes. The inflammation is caused by the hepatocytes replicating and releasing more hepatitis virus. Signs and symptoms include malaise, anorexia, loss of appetite, dark urine, pain in the upper right quadrant of the abdomen, vomiting, nausea, diarrhea, joint pain, and gray stool. Additionally, when the liver is diseased or injured, it is unable to break down hemoglobin effectively, and bilirubin can build up in the body, giving the skin and mucous membranes a yellowish color, a condition called **jaundice** ([\[link\]](#)). In severe cases, death from liver necrosis may occur.



(a) Hepatitis is inflammation of the liver resulting from a variety of root causes. It can cause jaundice. (b) Jaundice is characterized by yellowing of the skin, mucous membranes, and sclera of the eyes. (credit b left: modification of work by James Heilman, MD; credit b right: modification of work by “Sab3el3eish”/Wikimedia Commons)

Despite having many similarities, each of the hepatitis viruses has its own unique characteristics. HAV is generally transmitted through the fecal-oral route, close personal contact, or exposure to contaminated water or food. Hepatitis A can develop after an incubation period of 15 to 50 days (the mean is 30). It is normally mild or even asymptomatic and is usually self-limiting within weeks to months. A more severe form, fulminant hepatitis, rarely occurs but has a high fatality rate of 70–80%. Vaccination is available and is recommended especially for children (between ages one and two), those traveling to countries with higher risk, those with liver disease and certain other conditions, and drug users.

Although HBV is associated with similar signs and symptoms, transmission and outcomes differ. This virus has a mean incubation period of 120 days and is generally associated with exposure to infectious blood or body fluids such as semen or saliva. Exposure can occur through skin puncture, across the placenta, or through mucosal contact, but it is not spread through casual contact such as hugging, hand holding, sneezing, or coughing, or even through breastfeeding or kissing. Risk of infection is greatest for those who

use intravenous drugs or who have sexual contact with an infected individual. Health-care workers are also at risk from needle sticks and other injuries when treating infected patients. The infection can become chronic and may progress to cirrhosis or liver failure. It is also associated with liver cancer. Chronic infections are associated with the highest mortality rates and are more common in infants. Approximately 90% of infected infants become chronic carriers, compared with only 6–10% of infected adults.

[[footnote](#)] Vaccination is available and is recommended for children as part of the standard vaccination schedule (one dose at birth and the second by 18 months of age) and for adults at greater risk (e.g., those with certain diseases, intravenous drug users, and those who have sex with multiple partners). Health-care agencies are required to offer the HBV vaccine to all workers who have occupational exposure to blood and/or other infectious materials.

Centers for Disease Control and Prevention. “The ABCs of Hepatitis.” Updated 2016.

<http://www.cdc.gov/hepatitis/resources/professionals/pdfs/abctable.pdf>.

HCV is often undiagnosed and therefore may be more widespread than is documented. It has a mean incubation period of 45 days and is transmitted through contact with infected blood. Although some cases are asymptomatic and/or resolve spontaneously, 75%–85% of infected individuals become chronic carriers. Nearly all cases result from parenteral transmission often associated with IV drug use or transfusions. The risk is greatest for individuals with past or current history of intravenous drug use or who have had sexual contact with infected individuals. It has also been spread through contaminated blood products and can even be transmitted through contaminated personal products such as toothbrushes and razors. New medications have recently been developed that show great effectiveness in treating HCV and that are tailored to the specific genotype causing the infection.

HDV is uncommon in the United States and only occurs in individuals who are already infected with HBV, which it requires for replication. Therefore, vaccination against HBV is also protective against HDV infection. HDV is transmitted through contact with infected blood.

HEV infections are also rare in the United States but many individuals have a positive antibody titer for HEV. The virus is most commonly spread by the fecal-oral route through food and/or water contamination, or person-to-person contact, depending on the genotype of the virus, which varies by location. There are four genotypes that differ somewhat in their mode of transmission, distribution, and other factors (for example, two are zoonotic and two are not, and only one causes chronic infection). Genotypes three and four are only transmitted through food, while genotypes one and two are also transmitted through water and fecal-oral routes. Genotype one is the only type transmitted person-to-person and is the most common cause of HEV outbreaks. Consumption of undercooked meat, especially deer or pork, and shellfish can lead to infection. Genotypes three and four are zoonoses, so they can be transmitted from infected animals that are consumed. Pregnant women are at particular risk. This disease is usually self-limiting within two weeks and does not appear to cause chronic infection.

General laboratory testing for hepatitis begins with blood testing to examine liver function ([\[link\]](#)). When the liver is not functioning normally, the blood will contain elevated levels of alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin, total bilirubin, serum albumin, serum total protein, and calculated globulin, albumin/globulin (A/G) ratio. Some of these are included in a complete metabolic panel (CMP), which may first suggest a possible liver problem and indicate the need for more comprehensive testing. A hepatitis virus serological test panel can be used to detect antibodies for hepatitis viruses A, B, C, and sometimes D. Additionally, other immunological and genomic tests are available.

Specific treatments other than supportive therapy, rest, and fluids are often not available for hepatitis virus infection, except for HCV, which is often self-limited. Immunoglobulins can be used prophylactically following possible exposure. Medications are also used, including interferon alpha 2b and antivirals (e.g., lamivudine, entecavir, adefovir, and telbivudine) for chronic infections. Hepatitis C can be treated with interferon (as monotherapy or combined with other treatments), protease inhibitors, and other antivirals (e.g., the polymerase inhibitor sofosbuvir). Combination

treatments are commonly used. Antiviral and immunosuppressive medications may be used for chronic cases of HEV. In severe cases, liver transplants may be necessary. Additionally, vaccines are available to prevent infection with HAV and HBV. The HAV vaccine is also protective against HEV. The HBV vaccine is also protective against HDV. There is no vaccine against HCV.

Note:

Learn more information about [hepatitis virus](#) infections.

Note:

- Why do the five different hepatitis viruses all cause similar signs and symptoms?

Note:

Preventing HBV Transmission in Health-Care Settings

Hepatitis B was once a leading on-the-job hazard for health-care workers. Many health-care workers over the years have become infected, some developing cirrhosis and liver cancer. In 1982, the CDC recommended that health-care workers be vaccinated against HBV, and rates of infection have declined since then. Even though vaccination is now common, it is not always effective and not all individuals are vaccinated. Therefore, there is still a small risk for infection, especially for health-care workers working with individuals who have chronic infections, such as drug addicts, and for those with higher risk of needle sticks, such as phlebotomists. Dentists are also at risk.

Health-care workers need to take appropriate precautions to prevent infection by HBV and other illnesses. Blood is the greatest risk, but other body fluids can also transmit infection. Damaged skin, as occurs with eczema or psoriasis, can also allow transmission. Avoiding contact with

body fluids, especially blood, by wearing gloves and face protection and using disposable syringes and needles reduce the risk of infection. Washing exposed skin with soap and water is recommended. Antiseptics may also be used, but may not help. Post-exposure treatment, including treatment with hepatitis B immunoglobulin (HBIG) and vaccination, may be used in the event of exposure to the virus from an infected patient. Detailed protocols are available for managing these situations. The virus can remain infective for up to seven days when on surfaces, even if no blood or other fluids are visible, so it is important to consider the best choices for disinfecting and sterilizing equipment that could potentially transmit the virus. The CDC recommends a solution of 10% bleach to disinfect surfaces.[\[footnote\]](#) Finally, testing blood products is important to reduce the risk of transmission during transfusions and similar procedures. Centers for Disease Control and Prevention. “Hepatitis B FAQs for Health Professionals.” Updated August 4, 2016. <http://www.cdc.gov/hepatitis/HBV/HBVfaq.htm>.

Note:

Viral Hepatitis

Hepatitis involves inflammation of the liver that typically manifests with signs and symptoms such as jaundice, nausea, vomiting, joint pain, gray stool, and loss of appetite. However, the severity and duration of the disease can vary greatly depending on the causative agent. Some infections may be completely asymptomatic, whereas others may be life threatening. The five different viruses capable of causing hepatitis are compared in [\[link\]](#). For the sake of comparison, this table presents only the unique aspects of each form of viral hepatitis, not the commonalities.

Viral Forms of Hepatitis					
Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs	Vaccine
Hepatitis A	Hepatitisvirus A (HAV)	Usually asymptomatic or mild and self-limiting within one to two weeks to a few months, sometimes longer but not, chronic; in rare cases leads to serious or fatal fulminant hepatitis	Contaminated food, water, objects, and person to person	None	Vaccine recommended for one year olds and high-risk adults
Hepatitis B	Hepatitisvirus B (HBV)	Similar to Hepatitis A, but may progress to cirrhosis and liver failure; associated with liver cancer	Contact with infected body fluids (blood, semen, saliva), e.g., via IV drug use, sexual transmission, health-care workers treating infected patients	Interferon, entecavir, tenofovir, lamivudine, adefovir	Vaccine recommended for infants and high-risk adults
Hepatitis C	Hepatitisvirus C (HCV)	Often asymptomatic, with 75%–85% chronic carriers; may progress to cirrhosis and liver failure; associated with liver cancer	Contact with infected body fluids, e.g., via IV drug use, transfusions, sexual transmission	Depends on genotype and on whether cirrhosis is present; interferons, new treatment such as simeprevir plus sofosbuvir, ombitasvir/paritaprevir/ritonavir and dasabuvir	None available
Hepatitis D	Hepatitisvirus D (HDV)	Similar to hepatitis B; usually self-limiting within one to two weeks but can become chronic or fulminant in rare cases	Contact with infected blood; infections can only occur in patients already infected with hepatitis B	None	Hepatitis B vaccine protects against HDV
Hepatitis E	Hepatitisvirus E (HEV)	Generally asymptomatic or mild and self-limiting; typically does not cause chronic disease	Fecal-oral route, often in contaminated water or undercooked meat; most common in developing countries	Supportive treatment; usually self-limiting, but some strains can become chronic; antiviral and immunosuppressive possible for chronic cases	Vaccine available in China only

Key Concepts and Summary

- Common viral causes of gastroenteritis include rotaviruses, noroviruses, and astroviruses.
- Hepatitis may be caused by several unrelated viruses: hepatitis viruses A, B, C, D, and E.
- The hepatitis viruses differ in their modes of transmission, treatment, and potential for chronic infection.

Multiple Choice

Exercise:

Problem:

Which form of hepatitisvirus can only infect an individual who is already infected with another hepatitisvirus?

- a. HDV
- b. HAV
- c. HBV
- d. HEV

Solution:

A

Exercise:

Problem:

Which cause of viral gastroenteritis commonly causes projectile vomiting?

- a. hepatitisvirus
- b. Astroviruses
- c. Rotavirus
- d. Noroviruses

Solution:

D

Fill in the Blank

Exercise:

Problem: Jaundice results from a buildup of _____.

Solution:

bilirubin

Short Answer

Exercise:

Problem:

Which forms of viral hepatitis are transmitted through the fecal-oral route?

Critical Thinking

Exercise:

Problem:

Based on what you know about HBV, what are some ways that its transmission could be reduced in a health-care setting?

Protozoan Infections of the Gastrointestinal Tract

LEARNING OBJECTIVES

- Identify the most common protozoans that can cause infections of the GI tract
- Compare the major characteristics of specific protozoan diseases affecting the GI tract

Like other microbes, protozoa are abundant in natural microbiota but can also be associated with significant illness. Gastrointestinal diseases caused by protozoa are generally associated with exposure to contaminated food and water, meaning that those without access to good sanitation are at greatest risk. Even in developed countries, infections can occur and these microbes have sometimes caused significant outbreaks from contamination of public water supplies.

Giardiasis

Also called backpacker's diarrhea or beaver fever, **giardiasis** is a common disease in the United States caused by the flagellated protist *Giardia lamblia*, also known as *Giardia intestinalis* or *Giardia duodenalis* ([\[link\]](#)). To establish infection, *G. lamblia* uses a large adhesive disk to attach to the intestinal mucosa. The disk is comprised of microtubules. During adhesion, the flagella of *G. lamblia* move in a manner that draws fluid out from under the disk, resulting in an area of lower pressure that promotes its adhesion to the intestinal epithelial cells. Due to its attachment, *Giardia* also blocks absorption of nutrients, including fats.

Transmission occurs through contaminated food or water or directly from person to person. Children in day-care centers are at risk due to their tendency to put items into their mouths that may be contaminated. Large outbreaks may occur if a public water supply becomes contaminated. *Giardia* have a resistant cyst stage in their life cycle that is able to survive cold temperatures and the chlorination treatment typically used for drinking water in municipal reservoirs. As a result, municipal water must be filtered to trap and remove these cysts. Once consumed by the host, *Giardia* develops into the active trophozoite.

Infected individuals may be asymptomatic or have gastrointestinal signs and symptoms, sometimes accompanied by weight loss. Common symptoms, which appear one to three weeks after exposure, include diarrhea, nausea, stomach cramps, gas, greasy stool (because fat absorption is being blocked), and possible dehydration. The parasite remains in the colon and does not cause systemic infection. Signs and symptoms generally clear within two to six weeks. Chronic infections may develop and are often resistant to treatment. These are associated with weight loss, episodic diarrhea, and malabsorption syndrome due to the blocked nutrient absorption.

Diagnosis may be made using observation under the microscope. A stool ova and parasite (O&P) exam involves direct examination of a stool sample for the presence of cysts and trophozoites; it can be used to distinguish common parasitic intestinal infections. ELISA and other immunoassay tests, including commercial direct fluorescence antibody kits, are also used. The most common treatments use metronidazole as the first-line choice, followed by tinidazole. If the infection becomes chronic, the parasites may become resistant to medications.

Cryptosporidiosis

Another protozoan intestinal illness is **cryptosporidiosis**, which is usually caused by *Cryptosporidium parvum* or *C. hominis*. ([link](#)) These pathogens are commonly found in animals and can be spread in feces from mice, birds, and farm animals. Contaminated water and food are most commonly

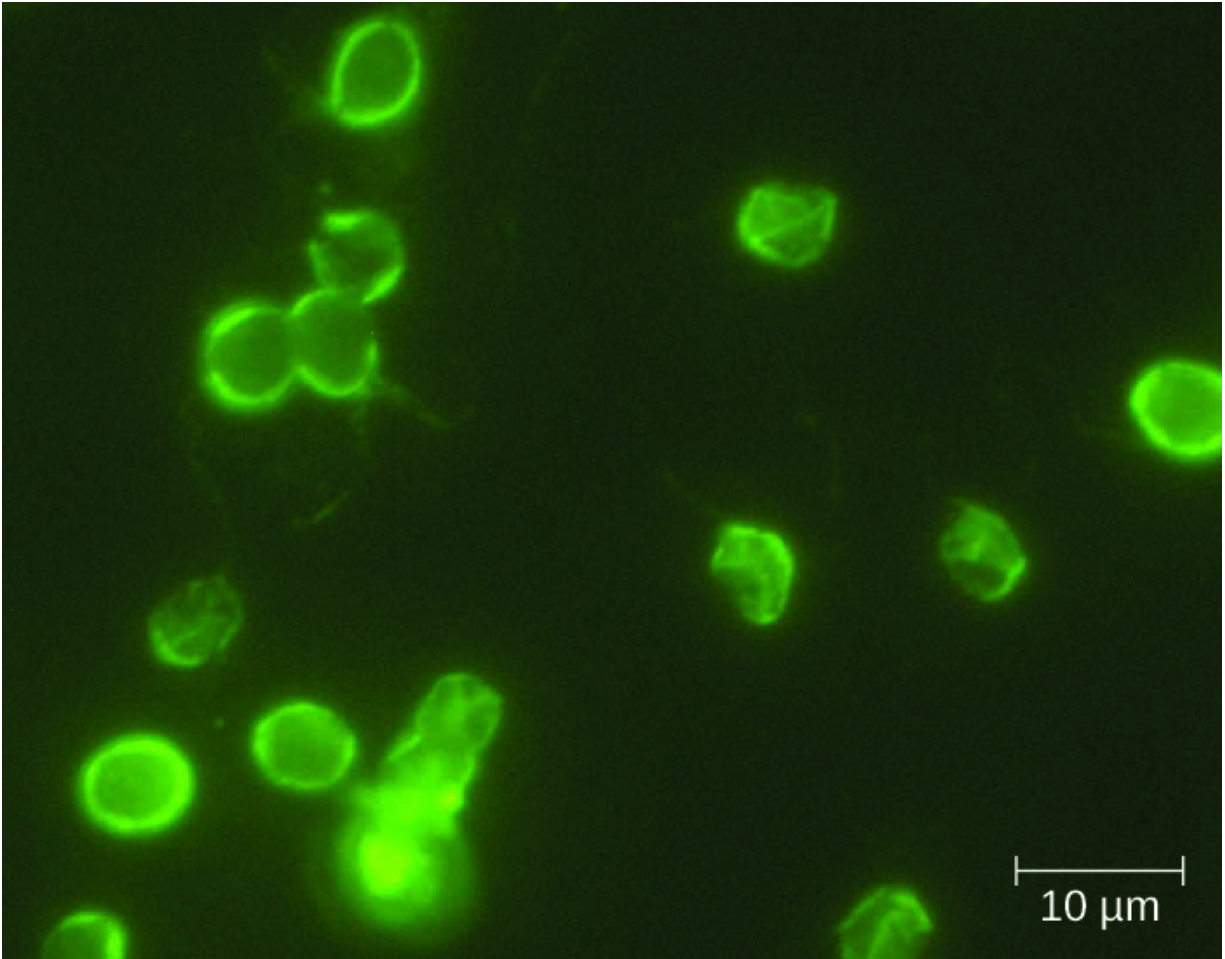
responsible for transmission. The protozoan can also be transmitted through human contact with infected animals or their feces.

In the United States, outbreaks of cryptosporidiosis generally occur through contamination of the public water supply or contaminated water at water parks, swimming pools, and day-care centers. The risk is greatest in areas with poor sanitation, making the disease more common in developing countries.

Signs and symptoms include watery diarrhea, nausea, vomiting, cramps, fever, dehydration, and weight loss. The illness is generally self-limiting within a month. However, immunocompromised patients, such as those with HIV/AIDS, are at particular risk of severe illness or death.

Diagnosis involves direct examination of stool samples, often over multiple days. As with giardiasis, a stool O&P exam may be helpful. Acid fast staining is often used. Enzyme immunoassays and molecular analysis (PCR) are available.

The first line of treatment is typically oral rehydration therapy. Medications are sometimes used to treat the diarrhea. The broad-range anti-parasitic drug nitazoxanide can be used to treat cryptosporidiosis. Other anti-parasitic drugs that can be used include azithromycin and paromomycin.



Immunofluorescent staining allows for visualization of *Cryptosporidium* spp. (credit: modification of work by EPA/H.D.A. Lindquist)

Amoebiasis (Amebiasis)

The protozoan parasite *Entamoeba histolytica* causes **amoebiasis**, which is known as **amoebic dysentery** in severe cases. *E. histolytica* is generally transmitted through water or food that has fecal contamination. The disease is most widespread in the developing world and is one of the leading causes of mortality from parasitic disease worldwide. Disease can be caused by as few as 10 cysts being transmitted.

Signs and symptoms range from nonexistent to mild diarrhea to severe amoebic dysentery. Severe infection causes the abdomen to become distended and may be associated with fever. The parasite may live in the colon without causing signs or symptoms or may invade the mucosa to cause colitis. In some cases, the disease spreads to the spleen, brain, genitourinary tract, or lungs. In particular, it may spread to the liver and cause an abscess. When a liver abscess develops, fever, nausea, liver tenderness, weight loss, and pain in the right abdominal quadrant may occur. Chronic infection may occur and is associated with intermittent diarrhea, mucus, pain, flatulence, and weight loss.

Direct examination of fecal specimens may be used for diagnosis. As with cryptosporidiosis, samples are often examined on multiple days. A stool O&P exam of fecal or biopsy specimens may be helpful. Immunoassay, serology, biopsy, molecular, and antibody detection tests are available. Enzyme immunoassay may not distinguish current from past illness. Magnetic resonance imaging (MRI) can be used to detect any liver abscesses. The first line of treatment is metronidazole or tinidazole, followed by diloxanide furoate, iodoquinol, or paromomycin to eliminate the cysts that remain.

Cyclosporiasis

The intestinal disease **cyclosporiasis** is caused by the protozoan *Cyclospora cayetanensis*. It is endemic to tropical and subtropical regions and therefore uncommon in the United States, although there have been outbreaks associated with contaminated produce imported from regions where the protozoan is more common.

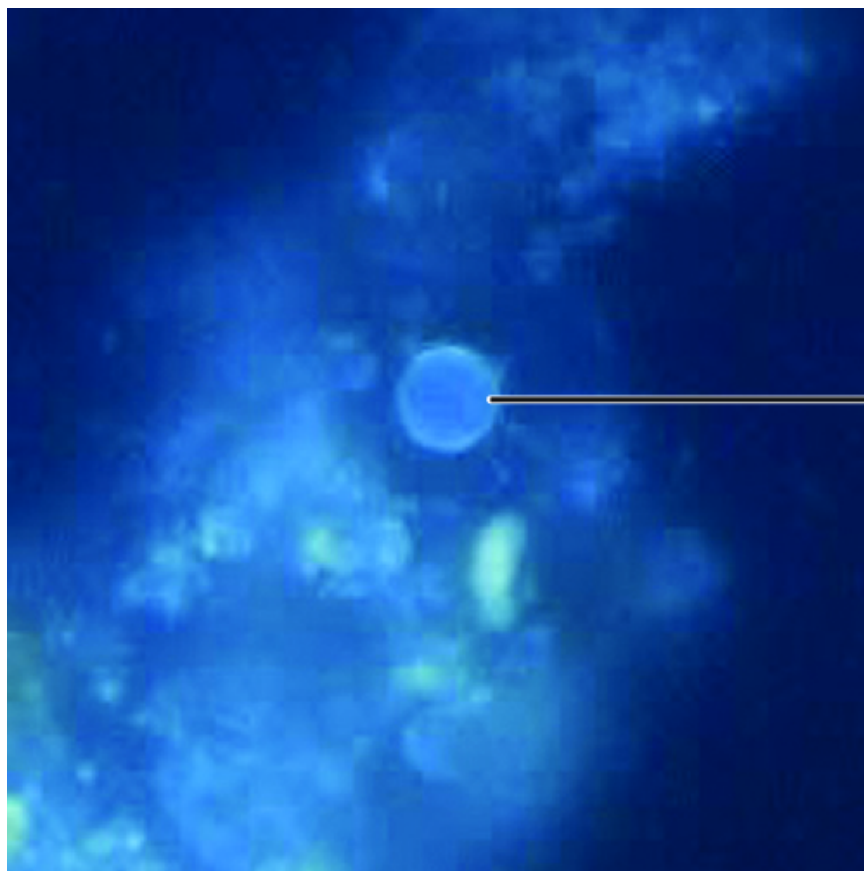
This protist is transmitted through contaminated food and water and reaches the lining of the small intestine, where it causes infection. Signs and symptoms begin within seven to ten days after ingestion. Based on limited data, it appears to be seasonal in ways that differ regionally and that are poorly understood.[\[footnote\]](#)

Centers for Disease Control and Prevention. “Cyclosporiasis FAQs for Health Professionals.” Updated June 13, 2014.

http://www.cdc.gov/parasites/cyclosporiasis/health_professionals/hp-faqs.html.

Some individuals do not develop signs or symptoms. Those who do may exhibit explosive and watery diarrhea, fever, nausea, vomiting, cramps, loss of appetite, fatigue, and bloating. These symptoms may last for months without treatment. Trimethoprim-sulfamethoxazole is the recommended treatment.

Microscopic examination is used for diagnosis. A stool O&P examination may be helpful. The oocysts have a distinctive blue halo when viewed using ultraviolet fluorescence microscopy ([link](#)).



C. cayetanensis

Cyclospora cayetanensis are autofluorescent under ultraviolet light.
(credit: modification of work by Centers for Disease Control and Prevention)

Note:

- Which protozoan GI infections are common in the United States?

Note:**Protozoan Gastrointestinal Infections**

Protozoan GI infections are generally transmitted through contaminated food or water, triggering diarrhea and vomiting that can lead to dehydration. Rehydration therapy is an important aspect of treatment, but most protozoan GI infections can also be treated with drugs that target protozoans.

Protozoan Infections of the GI Tract					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Amoebiasis (amoebic dysentery)	<i>Entamoeba histolytica</i>	From mild diarrhea to severe dysentery and colitis; may cause abscess on the liver	Fecal-oral route; ingestion of cysts from fecally contaminated water, food, or hands	Stool O&P exam, enzyme immunoassay	Metronidazole, tinidazole, diloxanide furoate, iodoquinol, paromomycin
Cryptosporidiosis	<i>Cryptosporidium parvum</i> , <i>Cryptosporidium hominis</i>	Watery diarrhea, nausea, vomiting, cramps, fever, dehydration, and weight loss	Contact with feces of infected mice, birds, farm animals; ingestion of contaminated food or water; exposure to contaminated water while swimming or bathing	Stool O&P exam, enzyme immunoassay, PCR	Nitazoxanide, azithromycin, and paromomycin
Cyclosporiasis	<i>Cyclospora cayetanensis</i>	Explosive diarrhea, fever, nausea, vomiting, cramps, loss of appetite, fatigue, bloating	Ingestion of contaminated food or water	Stool O&P exam using ultraviolet fluorescence microscopy	Trimethoprim-sulfamethoxazole
Giardiasis	<i>Giardia lamblia</i>	Diarrhea, nausea, stomach cramps, gas, greasy stool, dehydration if severe; sometimes malabsorption syndrome	Contact with infected individual or contaminated fomites; ingestion of contaminated food or water	Stool O&P exam; ELISA, direct fluorescence antibody assays	Metronidazole, tinidazole

Key Concepts and Summary

- **Giardiasis, cryptosporidiosis, amoebiasis, and cyclosporiasis** are intestinal infections caused by protozoans.
- Protozoan intestinal infections are commonly transmitted through contaminated food and water.
- Treatment varies depending on the causative agent, so proper diagnosis is important.
- Microscopic examination of stool or biopsy specimens is often used in diagnosis, in combination with other approaches.

Multiple Choice

Exercise:

Problem:

Which protozoan is associated with the ability to cause severe dysentery?

- a. *Giardia lamblia*
- b. *Cryptosporidium hominis*
- c. *Cyclospora cayetanesis*
- d. *Entamoeba histolytica*

Solution:

D

Exercise:

Problem:

Which protozoan has a unique appearance, with a blue halo, when viewed using ultraviolet fluorescence microscopy?

- a. *Giardia lamblia*
- b. *Cryptosporidium hominis*
- c. *Cyclospora cayetanesis*
- d. *Entamoeba histolytica*

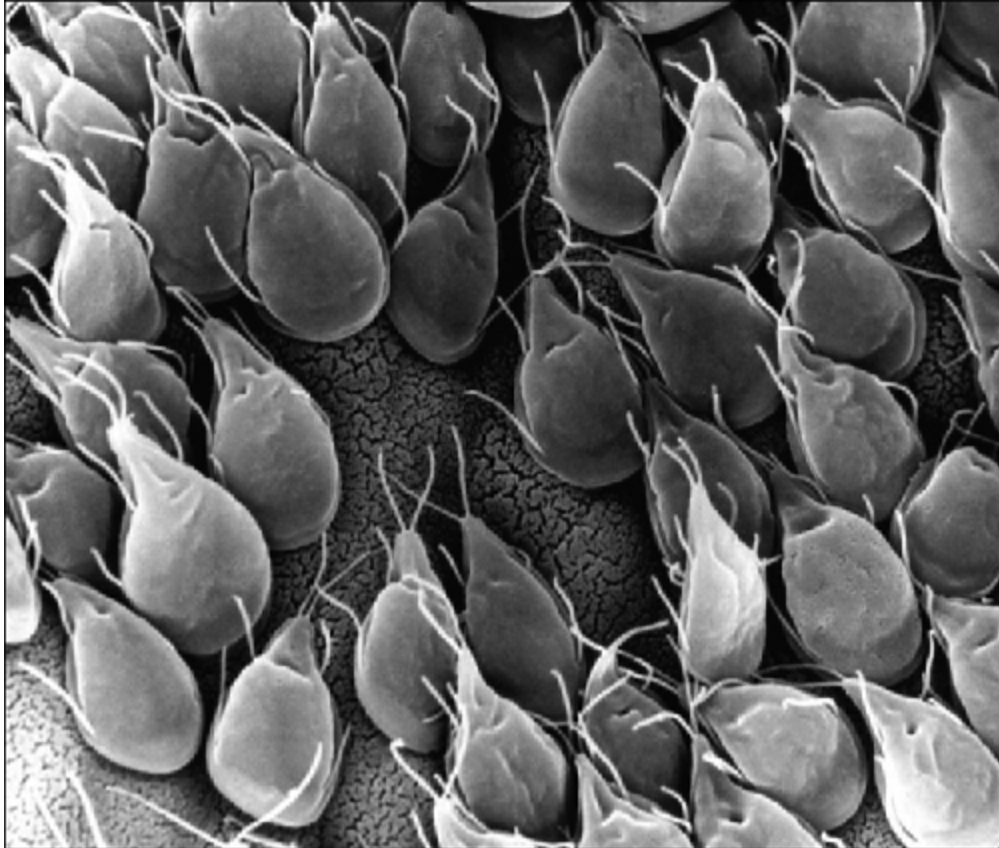
Solution:

C

Exercise:

Problem:

The micrograph shows protozoans attached to the intestinal wall of a gerbil. Based on what you know about protozoan intestinal parasites, what is it?



(credit: Dr. Stan Erlandsen, Centers for Disease Control and Prevention)

- a. *Giardia lamblia*
- b. *Cryptosporidium hominis*
- c. *Cyclospora cayetanesis*
- d. *Entamoeba histolytica*

Fill in the Blank

Exercise:

Problem:

Chronic _____ infections cause the unique sign of disease of greasy stool and are often resistant to treatment.

Solution:

giardia

Short Answer

Exercise:

Problem: What is an O&P exam?

Helminthic Infections of the Gastrointestinal Tract

LEARNING OBJECTIVES

- Identify the most common helminths that cause infections of the GI tract
- Compare the major characteristics of specific helminthic diseases affecting GI tract

Helminths are widespread intestinal parasites. These parasites can be divided into three common groups: round-bodied worms also described as nematodes, flat-bodied worms that are segmented (also described as cestodes), and flat-bodied worms that are non-segmented (also described as trematodes). The nematodes include roundworms, pinworms, hookworms, and whipworms. Cestodes include beef, pork, and fish tapeworms. Trematodes are collectively called flukes and more uniquely identified with the body site where the adult flukes are located. Although infection can have serious consequences, many of these parasites are so well adapted to the human host that there is little obvious disease.

Ascariasis

Infections caused by the large nematode roundworm *Ascaris lumbricoides*, a soil-transmitted helminth, are called **ascariasis**. Over 800 million to 1 billion people are estimated to be infected worldwide.[\[footnote\]](#) Infections are most common in warmer climates and at warmer times of year. At present, infections are uncommon in the United States. The eggs of the worms are transmitted through contaminated food and water. This may

happen if food is grown in contaminated soil, including when manure is used as fertilizer.

Centers for Disease Control and Prevention. “Parasites–Ascariasis.”

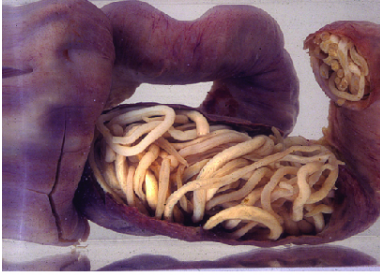
Updated May 24, 2016. <http://www.cdc.gov/parasites/ascariasis/index.html>.

When an individual consumes embryonated eggs (those with a developing embryo), the eggs travel to the intestine and the larvae are able to hatch. *Ascaris* is able to produce proteases that allow for penetration and degradation of host tissue. The juvenile worms can then enter the circulatory system and migrate to the lungs where they enter the alveoli (air sacs). From here they crawl to the pharynx and then follow the gut lumen to return to the small intestine, where they mature into adult roundworms. Females in the host will produce and release eggs that leave the host via feces. In some cases, the worms can block ducts such as those of the pancreas or gallbladder.

The infection is commonly asymptomatic. When signs and symptoms are present, they include shortness of breath, cough, nausea, diarrhea, blood in the stool, abdominal pain, weight loss, and fatigue. The roundworms may be visible in the stool. In severe cases, children with substantial infections may experience intestinal blockage.

The eggs can be identified by microscopic examination of the stool ([\[link\]](#)). In some cases, the worms themselves may be identified if coughed up or excreted in stool. They can also sometimes be identified by X-rays, ultrasounds, or MRIs.

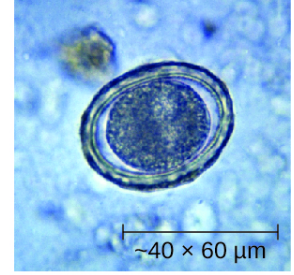
Ascariasis is self-limiting, but can last one to two years because the worms can inhibit the body’s inflammatory response through glycan gimmickry (see [Virulence Factors of Eukaryotic Pathogens](#)). The first line of treatment is mebendazole or albendazole. In some severe cases, surgery may be required.



(a)



(b)



(c)

(a) Adult *Ascaris lumbricoides* roundworms can cause intestinal blockage. (b) This mass of *A. lumbricoides* worms was excreted by a child. (c) A micrograph of a fertilized egg of *A. lumbricoides*. Fertilized eggs can be distinguished from unfertilized eggs because they are round rather than elongated and have a thicker cell wall. (credit a: modification of work by South African Medical Research Council; credit b: modification of work by James Gathany, Centers for Disease Control and Prevention; credit c: modification of work by Centers for Disease Control and Prevention)

Note:

- Describe the route by which *A. lumbricoides* reaches the host's intestines as an adult worm.

Hookworm

Two species of nematode worms are associated with **hookworm infection**. Both species are found in the Americas, Africa, and Asia. *Necator americanus* is found predominantly in the United States and Australia. Another species, *Ancylostoma doudenale*, is found in southern Europe, North Africa, the Middle East, and Asia.

The eggs of these species develop into larvae in soil contaminated by dog or cat feces. These larvae can penetrate the skin. After traveling through the venous circulation, they reach the lungs. When they are coughed up, they are then swallowed and can enter the intestine and develop into mature adults. At this stage, they attach to the wall of the intestine, where they feed on blood and can potentially cause anemia. Signs and symptoms include cough, an itchy rash, loss of appetite, abdominal pain, and diarrhea. In children, hookworms can affect physical and cognitive growth.

Some hookworm species, such as *Ancylostoma braziliense* that is commonly found in animals such as cats and dogs, can penetrate human skin and migrate, causing cutaneous larva migrans, a skin disease caused by the larvae of hookworms. As they move across the skin, in the subcutaneous tissue, pruritic tracks appear ([\[link\]](#)).

The infection is diagnosed using microscopic examination of the stool, allowing for observation of eggs in the feces. Medications such as albendazole, mebendazole, and pyrantel pamoate are used as needed to treat systemic infection. In addition to systemic medication for symptoms associated with cutaneous larva migrans, topical thiabendazole is applied to the affected areas.



(a)



(b)



(c)

(a) This animal hookworm, *Ancylostoma caninum*, is attached to the intestinal wall. (b) The tracks of hookworms are visible in this individual with cutaneous larva migrans. (c) This micrograph shows the microscopic egg of a hookworm. (credit a, c: modification of work by Centers for Disease Control and Prevention)

Strongyloidiasis

Strongyloidiasis is generally caused by *Strongyloides stercoralis*, a soil-transmitted helminth with both free-living and parasitic forms. In the parasitic form, the larvae of these nematodes generally penetrate the body through the skin, especially through bare feet, although transmission through organ transplantation or at facilities like day-care centers can also occur. When excreted in the stool, larvae can become free-living adults rather than developing into the parasitic form. These free-living worms reproduce, laying eggs that hatch into larvae that can develop into the parasitic form. In the parasitic life cycle, infective larvae enter the skin, generally through the feet. The larvae reach the circulatory system, which allows them to travel to the alveolar spaces of the lungs. They are transported to the pharynx where, like many other helminths, the infected patient coughs them up and swallows them again so that they return to the intestine. Once they reach the intestine, females live in the epithelium and produce eggs that develop asexually, unlike the free-living forms, which use sexual reproduction. The larvae may be excreted in the stool or can reinfect the host by entering the tissue of the intestines and skin around the anus, which can lead to chronic infections.

The condition is generally asymptomatic, although severe symptoms can develop after treatment with corticosteroids for asthma or chronic obstructive pulmonary disease, or following other forms of immunosuppression. When the immune system is suppressed, the rate of autoinfection increases, and huge amounts of larvae migrate to organs throughout the body.

Signs and symptoms are generally nonspecific. The condition can cause a rash at the site of skin entry, cough (dry or with blood), fever, nausea, difficulty breathing, bloating, pain, heartburn, and, rarely, arthritis, or cardiac or kidney complications. Disseminated strongyloidiasis or hyperinfection is a life-threatening form of the disease that can occur, usually following immunosuppression such as that caused by glucocorticoid

treatment (most commonly), with other immunosuppressive medications, with HIV infection, or with malnutrition.

As with other helminths, direct examination of the stool is important in diagnosis. Ideally, this should be continued over seven days. Serological testing, including antigen testing, is also available. These can be limited by cross-reactions with other similar parasites and by the inability to distinguish current from resolved infection. Ivermectin is the preferred treatment, with albendazole as a secondary option.

Note:

- How does an acute infection of *S. stercoralis* become chronic?

Pinworms (Enterobiasis)

Enterobius vermicularis, commonly called pinworms, are tiny (2–13 mm) nematodes that cause **enterobiasis**. Of all helminthic infections, enterobiasis is the most common in the United States, affecting as many as one-third of American children.[\[footnote\]](#) Although the signs and symptoms are generally mild, patients may experience abdominal pain and insomnia from itching of the perianal region, which frequently occurs at night when worms leave the anus to lay eggs. The itching contributes to transmission, as the disease is transmitted through the fecal-oral route. When an infected individual scratches the anal area, eggs may get under the fingernails and later be deposited near the individual's mouth, causing reinfection, or on fomites, where they can be transferred to new hosts. After being ingested, the larvae hatch within the small intestine and then take up residence in the colon and develop into adults. From the colon, the female adult exits the body at night to lay eggs ([\[link\]](#)).

“Roundworms.” *University of Maryland Medical Center Medical Reference Guide*. Last reviewed December 9, 2014.

<https://umm.edu/health/medical/altmed/condition/roundworms>.

Infection is diagnosed in any of three ways. First, because the worms emerge at night to lay eggs, it is possible to inspect the perianal region for worms while an individual is asleep. An alternative is to use transparent tape to remove eggs from the area around the anus first thing in the morning for three days to yield eggs for microscopic examination. Finally, it may be possible to detect eggs through examination of samples from under the fingernails, where eggs may lodge due to scratching. Once diagnosis has been made, mebendazole, albendazole, and pyrantel pamoate are effective for treatment.



(a)



(b)

(a) *E. vermicularis* are tiny nematodes commonly called pinworms. (b) This micrograph shows pinworm eggs.

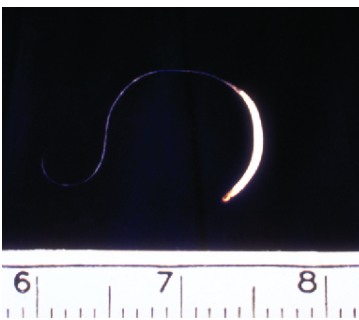
Trichuriasis

The nematode whipworm *Trichuris trichiura* is a parasite that is transmitted by ingestion from soil-contaminated hands or food and causes **trichuriasis**. Infection is most common in warm environments, especially when there is poor sanitation and greater risk of fecal contamination of soil, or when food is grown in soil using manure as a fertilizer. The signs and symptoms may be minimal or nonexistent. When a substantial infection develops, signs and symptoms include painful, frequent diarrhea that may contain mucus and blood. It is possible for the infection to cause rectal prolapse, a condition in which a portion of the rectum becomes detached from the inside of the body

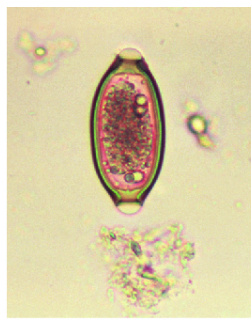
and protrudes from the anus ([\[link\]](#)). Severely infected children may experience reduced growth and their cognitive development may be affected.

When fertilized eggs are ingested, they travel to the intestine and the larvae emerge, taking up residence in the walls of the colon and cecum. They attach themselves with part of their bodies embedded in the mucosa. The larvae mature and live in the cecum and ascending colon. After 60 to 70 days, females begin to lay 3000 to 20,000 eggs per day.

Diagnosis involves examination of the feces for the presence of eggs. It may be necessary to use concentration techniques and to collect specimens on multiple days. Following diagnosis, the infection may be treated with mebendazole, albendazole, or ivermectin.



(a)



(b)



(c)

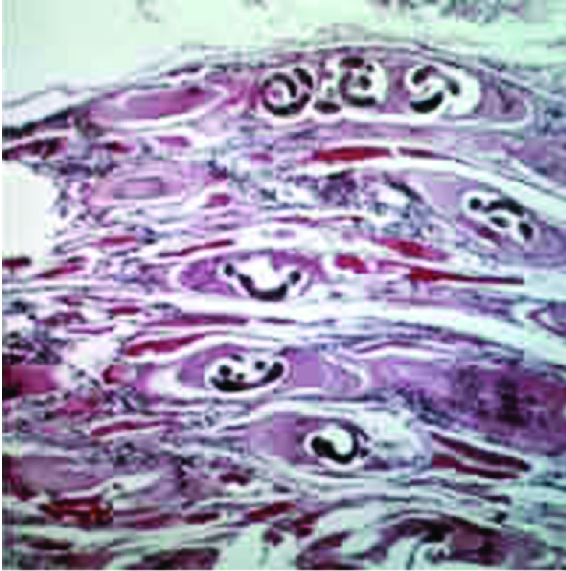
(a) This adult female *Trichuris* whipworm is a soil-transmitted parasite. (b) *Trichuris trichiura* eggs are ingested and travel to the intestines where the larvae emerge and take up residence. (c) Rectal prolapse is a condition that can result from whipworm infections. It occurs when the rectum loses its attachment to the internal body structure and protrudes from the anus. (credit a, b, c: modification of work by Centers for Disease Control and Prevention)

Trichinosis

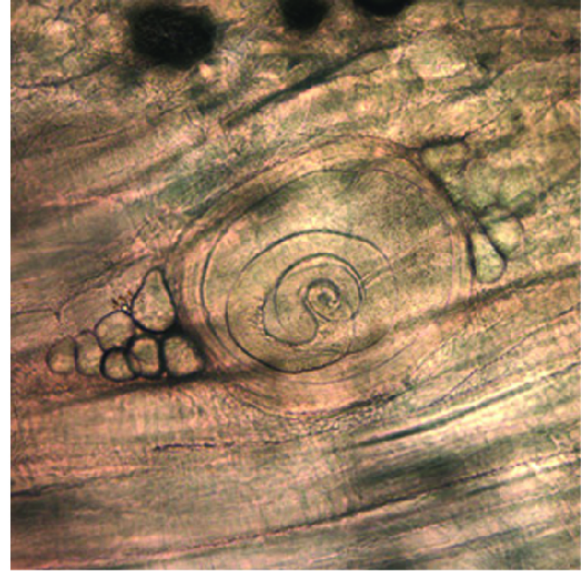
Trichinosis (trichenellosis) develops following consumption of food that contains *Trichinella spiralis* (most commonly) or other *Trichinella* species. These microscopic nematode worms are most commonly transmitted in meat, especially pork, that has not been cooked thoroughly. *T. spiralis* larvae in meat emerge from cysts when exposed to acid and pepsin in the stomach. They develop into mature adults within the large intestine. The larvae produced in the large intestine are able to migrate into the muscles mechanically via the stylet of the parasite, forming cysts. Muscle proteins are reduced in abundance or undetectable in cells that contain *Trichinella* (nurse cells). Animals that ingest the cysts from other animals can later develop infection ([\[link\]](#)).

Although infection may be asymptomatic, symptomatic infections begin within a day or two of consuming the nematodes. Abdominal symptoms arise first and can include diarrhea, constipation, and abdominal pain. Other possible symptoms include headache, light sensitivity, muscle pain, fever, cough, chills, and conjunctivitis. More severe symptoms affecting motor coordination, breathing, and the heart sometimes occur. It may take months for the symptoms to resolve, and the condition is occasionally fatal. Mild cases may be mistaken for influenza or similar conditions.

Infection is diagnosed using clinical history, muscle biopsy to look for larvae, and serological testing, including immunoassays. Enzyme immunoassay is the most common test. It is difficult to effectively treat larvae that have formed cysts in the muscle, although medications may help. It is best to begin treatment as soon as possible because medications such as mebendazole and albendazole are effective in killing only the adult worms in the intestine. Steroids may be used to reduce inflammation if larvae are in the muscles.



(a)



(b)

(a) This image shows larvae of *T. spiralis* within muscle. (b) In meat, the larvae have a characteristic coiled appearance, as seen in this partially digested larva in bear meat. (credit a, b: modification of work by Centers for Disease Control and Prevention)

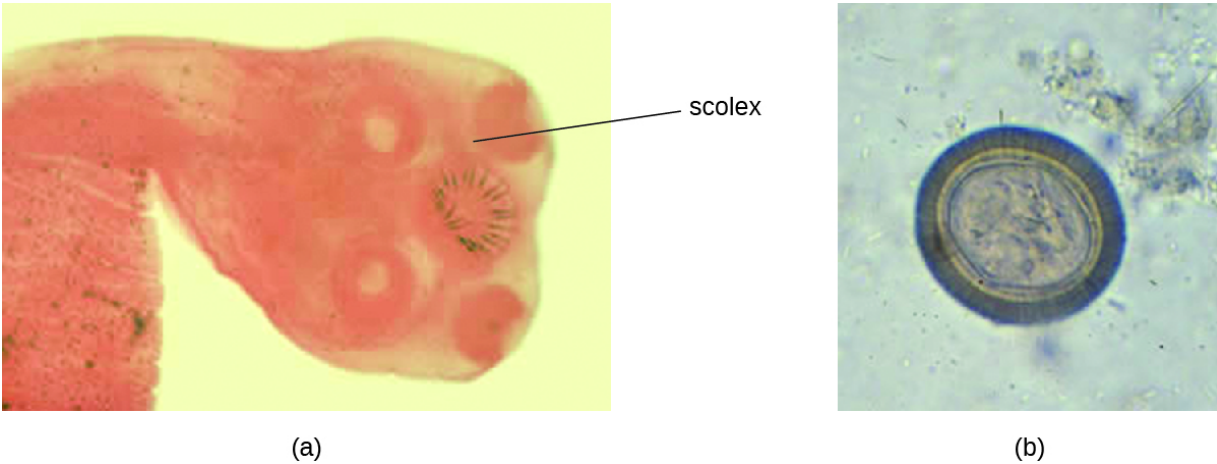
Note:

- Compare and contrast the transmissions of pinworms and whipworms.

Tapeworms (Taeniasis)

Taeniasis is a tapeworm infection, generally caused by pork (*Taenia solium*), beef (*Taenia saginata*), and Asian (*Taenia asiatica*) tapeworms found in undercooked meat. Consumption of raw or undercooked fish, including contaminated sushi, can also result in infection from the fish tapeworm (*Diphyllobothrium latum*). Tapeworms are flatworms (cestodes)

with multiple body segments and a head called a scolex that attaches to the intestinal wall. Tapeworms can become quite large, reaching 4 to 8 meters long ([link]). [link] illustrates the life cycle of a tapeworm.



- (a) An adult tapeworm uses the scolex to attach to the intestinal wall.
(b) The egg of a pork tapeworm (*Taenia solium*) is visible in this micrograph. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Tapeworms attached to the intestinal wall produce eggs that are excreted in feces. After ingestion by animals, the eggs hatch and the larvae emerge. They may take up residence in the intestine, but can sometimes move to other tissues, especially muscle or brain tissue. When *T. solium* larvae form cysts in tissue, the condition is called cysticercosis. This occurs through ingestion of eggs via the fecal-oral route, not through consumption of undercooked meat. It can develop in the muscles, eye (ophthalmic cysticercosis), or brain (neurocysticercosis).

Infections may be asymptomatic or they may cause mild gastrointestinal symptoms such as epigastric discomfort, nausea, diarrhea, flatulence, or hunger pains. It is also common to find visible tapeworm segments passed in the stool. In cases of cysticercosis, symptoms differ depending upon

where the cysts become established. Neurocysticercosis can have severe, life-threatening consequences and is associated with headaches and seizures because of the presence of the tapeworm larvae encysted in the brain. Cysts in muscles may be asymptomatic, or they may be painful.

To diagnose these conditions, microscopic analysis of stool samples from three separate days is generally recommended. Eggs or body segments, called proglottids, may be visible in these samples. Molecular methods have been developed but are not yet widely available. Imaging, such as CT and MRI, may be used to detect cysts. Praziquantel or niclosamide are used for treatment.

Note:

What's in Your Sushi Roll?

As foods that contain raw fish, such as sushi and sashimi, continue to increase in popularity throughout the world, so does the risk of parasitic infections carried by raw or undercooked fish. *Diphyllobothrium* species, known as fish tapeworms, is one of the main culprits. Evidence suggests that undercooked salmon caused an increase in *Diphyllobothrium* infections in British Columbia in the 1970s and early 1980s. In the years since, the number of reported cases in the United States and Canada has been low, but it is likely that cases are underreported because the causative agent is not easily recognized.[\[footnote\]](#)

Nancy Craig. "Fish Tapeworm and Sushi." *Canadian Family Physician* 58 (2012) 6: pp. 654–658.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3374688/>.

Another illness transmitted in undercooked fish is herring worm disease, or anisakiasis, in which nematodes attach to the epithelium of the esophagus, stomach, or small intestine. Cases have increased around the world as raw fish consumption has increased.[\[footnote\]](#)

Centers for Disease Control and Prevention. "Anisakiasis FAQs." Updated November 12, 2012. <http://www.cdc.gov/parasites/anisakiasis/faqs.html>.

Although the message may be unpopular with sushi lovers, fish should be frozen or cooked before eating. The extremely low and high temperatures associated with freezing and cooking kill worms and larvae contained in

the meat, thereby preventing infection. Ingesting fresh, raw sushi may make for a delightful meal, but it also entails some risk.

Hydatid Disease

Another cestode, *Echinococcus granulosus*, causes a serious infection known as **hydatid disease (cystic echinococcosis)**. *E. granulosus* is found in dogs (the definitive host), as well as several intermediate hosts (sheep, pigs, goats, cattle). The cestodes are transmitted through eggs in the feces from infected animals, which can be an occupational hazard for individuals who work in agriculture.

Once ingested, *E. granulosus* eggs hatch in the small intestine and release the larvae. The larvae invade the intestinal wall to gain access to the circulatory system. They form hydatid cysts in internal organs, especially in the lungs and liver, that grow slowly and are often undetected until they become large. If the cysts burst, a severe allergic reaction (anaphylaxis) may occur.

Cysts present in the liver can cause enlargement of the liver, nausea, vomiting, right epigastric pain, pain in the right upper quadrant, and possible allergic signs and symptoms. Cysts in the lungs can lead to alveolar disease. Abdominal pain, weight loss, pain, and malaise may occur, and inflammatory processes develop.

E. granulosus can be detected through imaging (ultrasonography, CT, MRI) that shows the cysts. Serologic tests, including ELISA and indirect hemagglutinin tests, are used. Cystic disease is most effectively treated with surgery to remove cysts, but other treatments are also available, including chemotherapy with anti-helminthic drugs (albendazole or mebendazole).

Note:

- Describe the risks of the cysts associated with taeniasis and hydatid disease.

Flukes

Flukes are flatworms that have a leaflike appearance. They are a type of trematode worm, and multiple species are associated with disease in humans. The most common are liver flukes and intestinal flukes ([link](#)).



(a)



(b)

(a) A liver fluke infects the bile ducts. (b) An intestinal fluke infects the intestines. (credit a: modification of work by Shafiei R, Sarkari B, Sadjjadi SM, Mowlavi GR, and Moshfe A; credit b: modification of work by Georgia Division of Public Health)

Liver Flukes

The **liver flukes** are several species of trematodes that cause disease by interfering with the bile duct. Fascioliasis is caused by *Fasciola hepatica*

and *Fasciola gigantica* in contaminated raw or undercooked aquatic plants (e.g., watercress). In *Fasciola* infection, adult flukes develop in the bile duct and release eggs into the feces. Clonorchiasis is caused by *Clonorchis sinensis* in contaminated freshwater fish. Other flukes, such as *Opisthorchis viverrini* (found in fish) and *Opisthorchis felinus* (found in freshwater snails), also cause infections. Liver flukes spend part of their life cycle in freshwater snails, which serve as an intermediate host. Humans are typically infected after eating aquatic plants contaminated by the infective larvae after they have left the snail. Once they reach the human intestine, they migrate back to the bile duct, where they mature. The life cycle is similar for the other infectious liver flukes, (see [\[link\]](#)).

When *Fasciola* flukes cause acute infection, signs and symptoms include nausea, vomiting, abdominal pain, rash, fever, malaise, and breathing difficulties. If the infection becomes chronic, with adult flukes living in the bile duct, then cholangitis, cirrhosis, pancreatitis, cholecystitis, and gallstones may develop. Symptoms are similar for infections by other liver flukes. Cholangiocarcinoma can occur from *C. sinensis* infection. The *Opisthorchis* species can also be associated with cancer development.

Diagnosis is accomplished using patient history and examination of samples from feces or other samples (such as vomitus). Because the eggs may appear similar, immunoassay techniques are available that can help distinguish species. The preferred treatment for fascioliasis is triclabendazole. *C. sinensis* and *Opisthorchis* spp. infections are treated with praziquantel or albendazole.

Intestinal Flukes

The **intestinal flukes** are trematodes that develop in the intestines. Many, such as *Fasciolopsis buski*, which causes fasciolopsiasis, are closely related to liver flukes. Intestinal flukes are ingested from contaminated aquatic plants that have not been properly cooked. When the cysts are consumed, the larvae emerge in the duodenum and develop into adults while attached to the intestinal epithelium. The eggs are released in stool.

Intestinal fluke infection is often asymptomatic, but some cases may involve mild diarrhea and abdominal pain. More severe symptoms such as vomiting, nausea, allergic reactions, and anemia can sometimes occur, and high parasite loads may sometimes lead to intestinal obstructions.

Diagnosis is the same as with liver flukes: examination of feces or other samples and immunoassay. Praziquantel is used to treat infections caused by intestinal flukes.

Note:

- How are flukes transmitted?

Note:

Helminthic Gastrointestinal Infections

Numerous helminths are capable of colonizing the GI tract. Many such infections are asymptomatic, but others may cause signs and symptoms ranging from mild GI stress to severe systemic infection. Helminths have complex and unique life cycles that dictate their specific modes of transmission. Most helminthic infections can be treated with medications.

Common Helminthic Infections of the GI Tract

Disease	Causative Agent(s)	Mode of Transmission	Laboratory Tests	Symptoms	Treatments
Ascariasis	<i>Ascaris lumbricoides</i>	Eggs in fecally contaminated food or water	Microscopic examination of the stool, imaging	Shortness of breath, cough, nausea, diarrhea, blood in stool, abdominal pain, weight loss, fatigue	Self-limiting within 1 to 2 years; albendazole and mebendazole if needed
Hookworm	<i>Necator americanus</i> , <i>Ancylostoma douduale</i>	Larvae in soil contaminated by dog or cat feces penetrate skin	Microscopic examination of stool (may require a concentration procedure)	Cough, itchy rash, loss of appetite, abdominal pain, diarrhea; in children, may affect physical and cognitive growth	Albendazole and mebendazole; pyrantel pamoate may if needed
Strongyloidiasis	<i>Strongyloides stercoralis</i>	Soil-dwelling larvae penetrate the skin, usually bare feet	Microscopic examination of stool over several days (ideally at least 7); some serologic testing available	Often asymptomatic; cough (sometimes bloody), skin rash, abdominal pain, and diarrhea; in immunosuppressed patients, may become disseminated, causing serious and potentially fatal complications	Ivermectin (preferred), albendazole
Enterobiasis (pinworm)	<i>Enterobius vermicularis</i>	Fecal–oral route	Observation of eggs or worms from anal area; examination of samples under fingernails	Itching around the anus, abdominal pain, insomnia, irritation of female genital tract	Mebendazole, albendazole, pyrantel pamoate
Trichiuriasis (whipworm)	<i>Trichuris trichiura</i>	Fecal contamination or fertilization in soil	Microscopic examination of stool	Abdominal pain, anemia, diarrhea that may be bloody	Albendazole, mebendazole, ivermectin if needed
Trichinosis	<i>Trichinella spiralis</i>	Eating raw or undercooked pork or other meat of infected animal	Clinical history, muscle biopsy, serological testing, enzyme immunoassay	Diarrhea, constipation, abdominal pain, headache, cough, chills, light sensitivity, muscle pain, fever, conjunctivitis; in severe cases may affect motor coordination, breathing, heart function	Albendazole, mebendazole if needed
Taeniasis and cysticercosis	<i>Taenia solium</i> , <i>T. saginata</i> , <i>T. asiatica</i> , <i>Diphyllobothrium latum</i>	Eating raw or undercooked beef or pork from infected animal	Observation of worm segments or microscopic eggs in stool samples	Asymptomatic or mild GI distress; cysts in muscle, eye, or brain (cysticercosis); brain cysts can cause headaches, seizures, or death	Praziquantel or niclosamide
Cystic echinococcosis (hydatid disease)	<i>Echinococcus granulosus</i> (cystic)	Exposure to eggs in feces of infected dogs or livestock	Imaging; serological testing including ELISA and indirect hemagglutinin test	Cysts in lungs, liver, and other organs causing nausea, GI distress, and weight loss; severe anaphylaxis or death if cysts burst	Surgical removal or aspiration of cysts or chemotherapy with albendazole or mebendazole
Liver fluke infections	<i>Fasciola hepatica</i> , <i>F. gigantica</i> , <i>Clonorchis sinensis</i> , <i>Opisthorchis viverrini</i> , <i>O. felineus</i>	Eating raw or undercooked aquatic plants (<i>Fasciola</i> spp.) or freshwater fish (<i>Clonorchis</i> spp.) contaminated with eggs or cysts	Microscopic examination of eggs in stool or other samples; immunoassays	Fever, malaise, anemia, abdominal symptoms, transaminitis; cholangitis, cirrhosis, pancreatitis, cholecystitis, gall stones in chronic phase	Triclabendazole (preferred) for <i>Fasciola</i> spp.; praziquantel and albendazole for <i>C. sinensis</i> and <i>Opisthorchis</i> spp.
Fasciolopiasis (intestinal fluke)	<i>Fasciola buski</i>	Eating raw or undercooked aquatic plants containing cysts	Microscopic examination of eggs in stool or other samples; immunoassays	Diarrhea, abdominal pain; in severe cases, vomiting, nausea, intestinal obstruction, anemia, allergic reactions	Praziquantel

Helminthic Infections of the GI Tract (continued)					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Strongyloidiasis	<i>Strongyloides stercoralis</i>	Often asymptomatic; cough (sometimes bloody), skin rash, abdominal pain, diarrhea; in immunosuppressed patients, may become disseminated, causing serious and potentially fatal complications	Soil-dwelling larvae penetrate the skin, usually bare feet	Microscopic observation of larvae in stool; serological testing for antigens	Ivermectin, albendazole
Tapeworms (taeniasis)	<i>Taenia solium</i> , <i>T. saginata</i> , <i>T. asiatica</i> , <i>Diphyllobothrium latum</i>	Asymptomatic or mild GI distress; cysts in muscle, eye, or brain (cysticercosis); brain cysts can cause headaches, seizures, or death	Ingestion of raw or undercooked pork or beef from infected animal	Observation of worm segments or microscopic eggs in stool; CT or MRI to detect cysts	Praziquantel, niclosamide
Trichinosis	<i>Trichinella spiralis</i> , other <i>Trichinella</i> spp.	Diarrhea, constipation, abdominal pain, headache, cough, chills, light sensitivity, muscle pain, fever, conjunctivitis; in severe cases may affect motor coordination, breathing, heart function	Ingestion of raw or undercooked pork or other meat of infected animal	Observation of cysts in muscle biopsy, enzyme immunoassay	Albendazole, mebendazole
Whipworm (trichuriasis)	<i>Trichuris trichiura</i>	Abdominal pain, anemia, diarrhea (possibly bloody), rectal prolapse	Ingestion of eggs in fecally contaminated food	Microscopic observation of eggs in stool	Albendazole, mebendazole, ivermectin

Note:

Resolution

Carli's doctor explained that she had bacterial gastroenteritis caused by *Salmonella* bacteria. The source of these bacteria was likely the undercooked egg. Had the egg been fully cooked, the high temperature would have been sufficient to kill any *Salmonella* in or on the egg. In this case, enough bacteria survived to cause an infection once the egg was eaten.

Carli's signs and symptoms continued to worsen. Her fever became higher, her vomiting and diarrhea continued, and she began to become dehydrated. She felt thirsty all the time and had continual abdominal cramps. Carli's

doctor treated her with intravenous fluids to help with her dehydration, but did not prescribe antibiotics. Carli's parents were confused because they thought a bacterial infection should always be treated with antibiotics. The doctor explained that the worst medical problem for Carli was dehydration. Except in the most vulnerable and sick patients, such as those with HIV/AIDS, antibiotics do not reduce recovery time or improve outcomes in *Salmonella* infections. In fact, antibiotics can actually delay the natural excretion of bacteria from the body. Rehydration therapy replenishes lost fluids, diminishing the effects of dehydration and improving the patient's condition while the infection resolves. After two days of rehydration therapy, Carli's signs and symptoms began to fade. She was still somewhat thirsty, but the amount of urine she passed became larger and the color lighter. She stopped vomiting. Her fever was gone, and so was the diarrhea. At that point, stool analysis found very few *Salmonella* bacteria. In one week, Carli was discharged as fully recovered. Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- Helminths often cause intestinal infections after transmission to humans through exposure to contaminated soil, water, or food. Signs and symptoms are often mild, but severe complications may develop in some cases.
- *Ascaris lumbricoides* eggs are transmitted through contaminated food or water and hatch in the intestine. Juvenile larvae travel to the lungs and then to the pharynx, where they are swallowed and returned to the intestines to mature. These nematode roundworms cause **ascariasis**.
- *Necator americanus* and *Ancylostoma doudenale* cause **hookworm infection** when larvae penetrate the skin from soil contaminated by dog or cat feces. They travel to the lungs and are then swallowed to mature in the intestines.
- *Strongyloides stercoralis* are transmitted from soil through the skin to the lungs and then to the intestine where they cause **strongyloidiasis**.
- *Enterobius vermicularis* are nematode pinworms transmitted by the fecal-oral route. After ingestion, they travel to the colon where they

cause **enterobiasis**.

- *Trichuris trichiura* can be transmitted through soil or fecal contamination and cause **trichuriasis**. After ingestion, the eggs travel to the intestine where the larvae emerge and mature, attaching to the walls of the colon and cecum.
- *Trichinella* spp. is transmitted through undercooked meat. Larvae in the meat emerge from cysts and mature in the large intestine. They can migrate to the muscles and form new cysts, causing **trichinosis**.
- *Taenia* spp. and *Diphyllobothrium latum* are tapeworms transmitted through undercooked food or the fecal-oral route. *Taenia* infections cause **taeniasis**. Tapeworms use their scolex to attach to the intestinal wall. Larvae may also move to muscle or brain tissue.
- *Echinococcus granulosus* is a cestode transmitted through eggs in the feces of infected animals, especially dogs. After ingestion, eggs hatch in the small intestine, and the larvae invade the intestinal wall and travel through the circulatory system to form dangerous cysts in internal organs, causing **hydatid disease**.
- Flukes are transmitted through aquatic plants or fish. **Liver flukes** cause disease by interfering with the bile duct. **Intestinal flukes** develop in the intestines, where they attach to the intestinal epithelium.

Multiple Choice

Exercise:

Problem: What is another name for *Trichuris trichiura*?

- a. pinworm
- b. whipworm
- c. hookworm
- d. ascariasis

Solution:

B

Exercise:**Problem:**

Which type of helminth infection can be diagnosed using tape?

- a. pinworm
- b. whipworm
- c. hookworm
- d. tapeworm

Solution:

A

Fill in the Blank**Exercise:**

Problem: Liver flukes are often found in the _____ duct.

Solution:

bile

Short Answer**Exercise:****Problem:**

Why does the coughing up of worms play an important part in the life cycle of some helminths, such as the roundworm *Ascaris lumbricoides*?

Critical Thinking

Exercise:

Problem:

Cases of strongyloidiasis are often more severe in patients who are using corticosteroids to treat another disorder. Explain why this might occur.

Introduction

class="introduction"

Yellow fever
is a viral
hemorrhagic
disease that
can cause
liver damage,
resulting in
jaundice (left)
as well as
serious and
sometimes
fatal
complications
. The virus
that causes
yellow fever
is transmitted
through the
bite of a
biological
vector, the
Aedes aegypti
mosquito
(right). (credit
left:
modification
of work by
Centers for
Disease
Control and
Prevention;
credit right:
modification
of work by

James
Gathany,
Centers for
Disease
Control and
Prevention)



Yellow fever was once common in the southeastern US, with annual outbreaks of more than 25,000 infections in New Orleans in the mid-1800s. [\[footnote\]](#) In the early 20th century, efforts to eradicate the virus that causes yellow fever were successful thanks to vaccination programs and effective control (mainly through the insecticide dichlorodiphenyltrichloroethane [DDT]) of *Aedes aegypti*, the mosquito that serves as a vector. Today, the virus has been largely eradicated in North America.

Centers for Disease Control and Prevention. “The History of Yellow Fever.” <http://www.cdc.gov/travel-training/local/HistoryEpidemiologyandVaccination/page27568.html>

Elsewhere, efforts to contain yellow fever have been less successful. Despite mass vaccination campaigns in some regions, the risk for yellow fever epidemics is rising in dense urban cities in Africa and South America. [\[footnote\]](#) In an increasingly globalized society, yellow fever could easily make a comeback in North America, where *A. aegypti* is still present. If these mosquitoes were exposed to infected individuals, new outbreaks would be possible.

C.L. Gardner, K.D. Ryman. "Yellow Fever: A Reemerging Threat." *Clinical Laboratory Medicine* 30 no. 1 (2010):237–260.

Like yellow fever, many of the circulatory and lymphatic diseases discussed in this chapter are emerging or re-emerging worldwide. Despite medical advances, diseases like malaria, Ebola, and others could become endemic in the US given the right circumstances.

Anatomy of the Circulatory and Lymphatic Systems

LEARNING OBJECTIVES

- Describe the major anatomical features of the circulatory and lymphatic systems
- Explain why the circulatory and lymphatic systems lack normal microbiota
- Explain how microorganisms overcome defenses of the circulatory and lymphatic systems to cause infection
- Describe general signs and symptoms of disease associated with infections of the circulatory and lymphatic systems

Note:

Part 1

Barbara is a 43-year-old patient who has been diagnosed with metastatic inflammatory breast cancer. To facilitate her ongoing chemotherapy, her physician implanted a port attached to a central venous catheter. At a recent checkup, she reported feeling restless and complained that the site of the catheter had become uncomfortable. After removing the dressing, the physician observed that the surgical site appeared red and was warm to the touch, suggesting a localized infection. Barbara's was also running a fever of 38.2 °C (100.8 °F). Her physician treated the affected area with a topical antiseptic and applied a fresh dressing. She also prescribed a course of the antibiotic oxacillin.

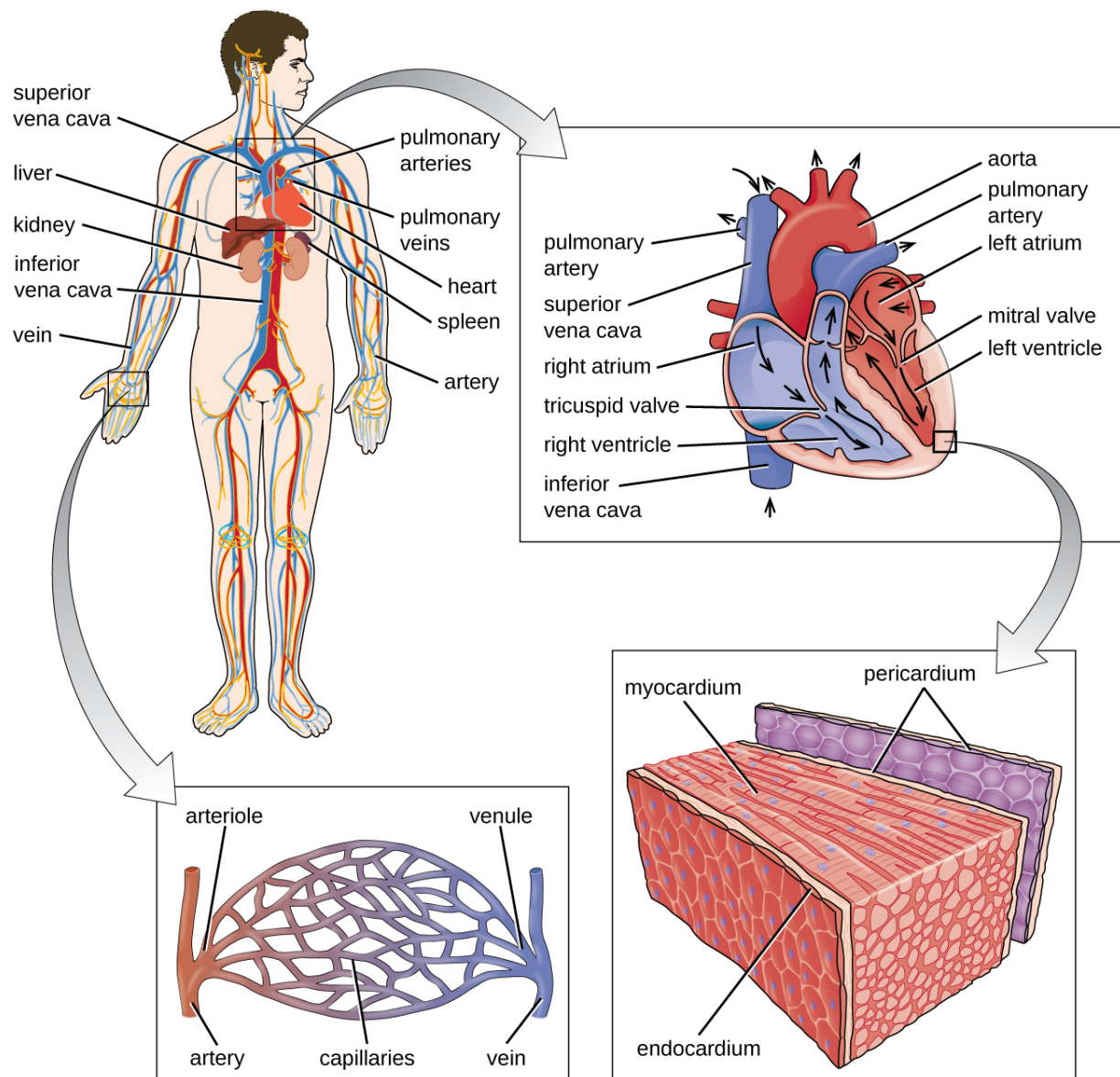
- Based on this information, what factors likely contributed to Barbara's condition?
- What is the most likely source of the microbes involved?

Jump to the [next](#) Clinical Focus box.

The circulatory and lymphatic systems are networks of vessels and a pump that transport blood and lymph, respectively, throughout the body. When these systems are infected with a microorganism, the network of vessels can facilitate the rapid dissemination of the microorganism to other regions of the body, sometimes with serious results. In this section, we will examine some of the key anatomical features of the circulatory and lymphatic systems, as well as general signs and symptoms of infection.

The Circulatory System

The circulatory (or cardiovascular) system is a closed network of organs and vessels that moves blood around the body ([\[link\]](#)). The primary purposes of the circulatory system are to deliver nutrients, immune factors, and oxygen to tissues and to carry away waste products for elimination. The heart is a four-chambered pump that propels the blood throughout the body. Deoxygenated blood enters the right atrium through the superior vena cava and the inferior vena cava after returning from the body. The blood next passes through the tricuspid valve to enter the right ventricle. When the heart contracts, the blood from the right ventricle is pumped through the pulmonary arteries to the lungs. There, the blood is oxygenated at the alveoli and returns to the heart through the pulmonary veins. The oxygenated blood is received at the left atrium and proceeds through the mitral valve to the left ventricle. When the heart contracts, the oxygenated blood is pumped throughout the body via a series of thick-walled vessels called arteries. The first and largest **artery** is called the aorta. The arteries sequentially branch and decrease in size (and are called arterioles) until they end in a network of smaller vessels called capillaries. The **capillary** beds are located in the interstitial spaces within tissues and release nutrients, immune factors, and oxygen to those tissues. The capillaries connect to a series of vessels called venules, which increase in size to form the **veins**. The veins join together into larger vessels as they transfer blood back to the heart. The largest veins, the superior and inferior vena cava, return the blood to the right atrium.



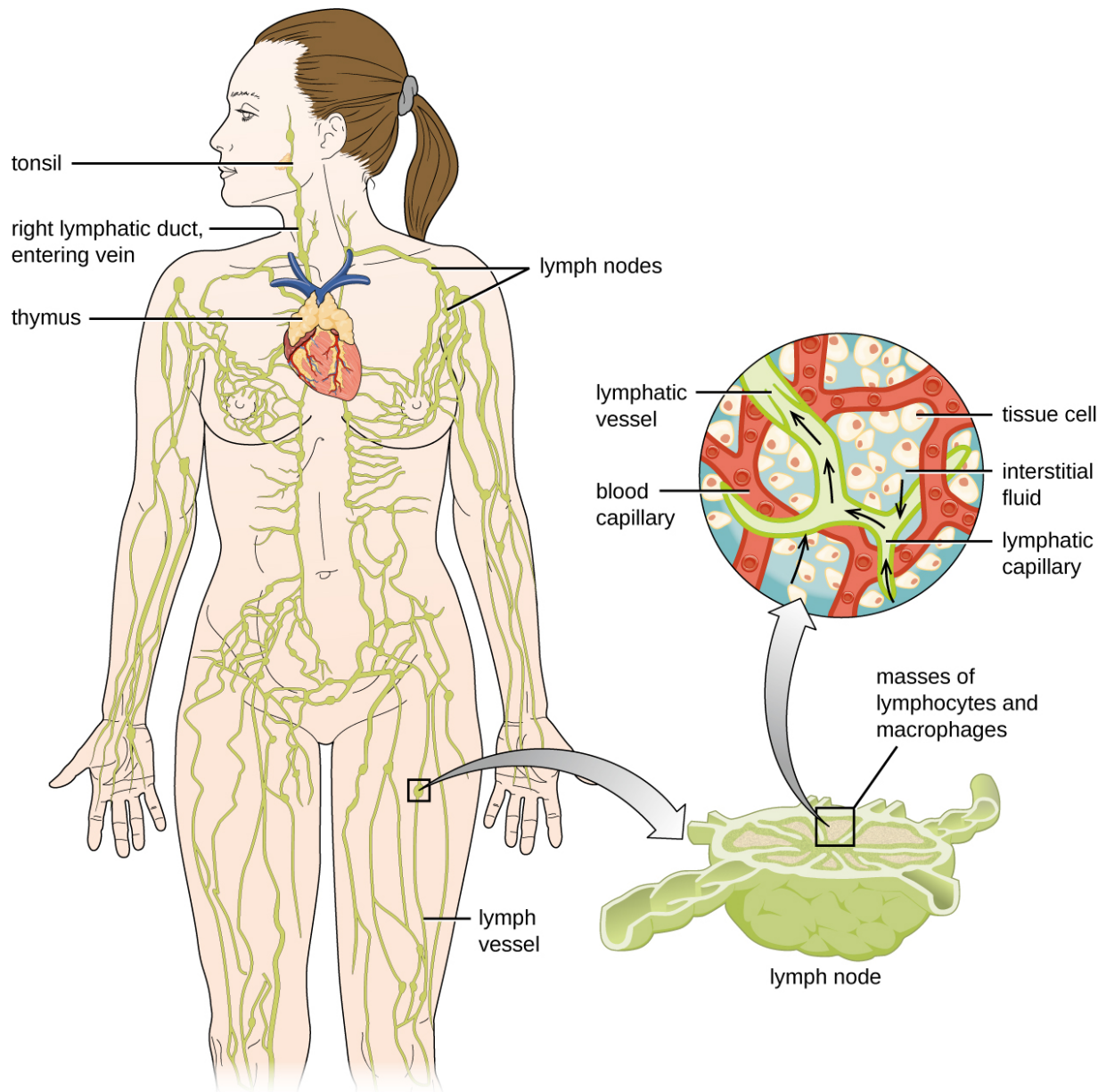
The major components of the human circulatory system include the heart, arteries, veins, and capillaries. This network delivers blood to the body's organs and tissues. (credit top left: modification of work by Mariana Ruiz Villareal; credit bottom right: modification of work by Bruce Blaus)

Other organs play important roles in the circulatory system as well. The kidneys filter the blood, removing waste products and eliminating them in

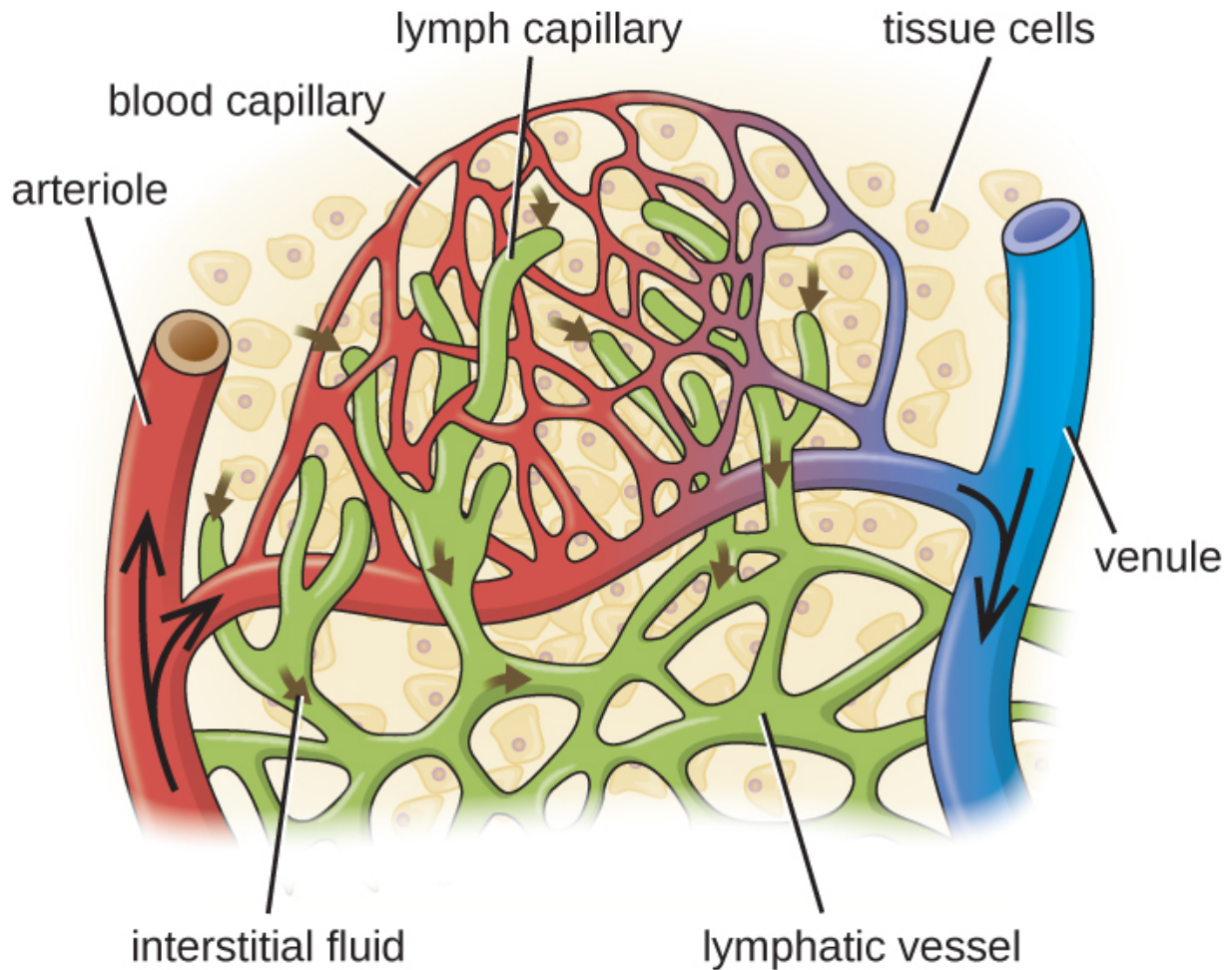
the urine. The liver also filters the blood and removes damaged or defective red blood cells. The spleen filters and stores blood, removes damaged red blood cells, and is a reservoir for immune factors. All of these filtering structures serve as sites for entrapment of microorganisms and help maintain an environment free of microorganisms in the blood.

The Lymphatic System

The lymphatic system is also a network of vessels that run throughout the body ([\[link\]](#)). However, these vessels do not form a full circulating system and are not pressurized by the heart. Rather, the lymphatic system is an open system with the fluid moving in one direction from the extremities toward two drainage points into veins just above the heart. Lymphatic fluids move more slowly than blood because they are not pressurized. Small lymph capillaries interact with blood capillaries in the interstitial spaces in tissues. Fluids from the tissues enter the lymph capillaries and are drained away ([\[link\]](#)). These fluids, termed lymph, also contain large numbers of white blood cells.



The essential components of the human lymphatic system drain fluid away from tissues.

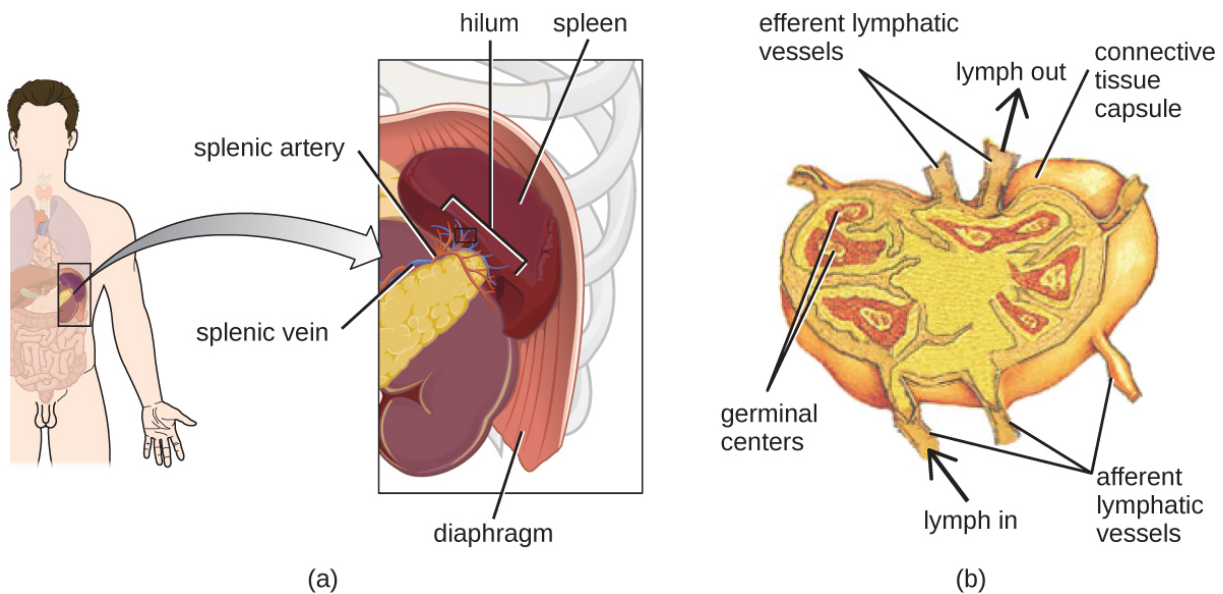


Blood enters the capillaries from an arteriole (red) and leaves through venules (blue). Interstitial fluids may drain into the lymph capillaries (green) and proceed to lymph nodes. (credit: modification of work by National Cancer Institute, National Institutes of Health)

The lymphatic system contains two types of lymphoid tissues. The **primary lymphoid tissue** includes bone marrow and the thymus. Bone marrow contains the hematopoietic stem cells (HSC) that differentiate and mature into the various types of blood cells and lymphocytes (see [\[link\]](#)). The **secondary lymphoid tissues** include the spleen, lymph nodes, and several areas of diffuse lymphoid tissues underlying epithelial membranes. The **spleen**, an encapsulated structure, filters blood and captures pathogens and

antigens that pass into it ([\[link\]](#)). The spleen contains specialized macrophages and dendritic cells that are crucial for antigen presentation, a mechanism critical for activation of T lymphocytes and B lymphocytes (see [Major Histocompatibility Complexes and Antigen-Presenting Cells](#)).

Lymph nodes are bean-shaped organs situated throughout the body. These structures contain areas called germinal centers that are rich in B and T lymphocytes. The **lymph nodes** also contain macrophages and dendritic cells for antigen presentation. Lymph from nearby tissues enters the lymph node through afferent lymphatic vessels and encounters these lymphocytes as it passes through; the lymph exits the lymph node through the efferent lymphatic vessels ([\[link\]](#)).



(a) The spleen is a lymphatic organ located in the upper left quadrant of the abdomen near the stomach and left kidney. It contains numerous phagocytes and lymphocytes that combat and prevent circulatory infections by killing and removing pathogens from the blood. (b) Lymph nodes are masses of lymphatic tissue located along the larger lymph vessels. They contain numerous lymphocytes that kill and remove pathogens from lymphatic fluid that drains from surrounding tissues.

Note:

The lymphatic system filters fluids that have accumulated in tissues before they are returned to the blood. A brief overview of this process is provided at [this](#) website.

Note:

- What is the main function of the lymphatic system?

Infections of the Circulatory System

Under normal circumstances, the circulatory system and the blood should be sterile; the circulatory system has no normal microbiota. Because the system is closed, there are no easy portals of entry into the circulatory system for microbes. Those that are able to breach the body's physical barriers and enter the bloodstream encounter a host of circulating immune defenses, such as antibodies, complement proteins, phagocytes, and other immune cells. Microbes often gain access to the circulatory system through a break in the skin (e.g., wounds, needles, intravenous catheters, insect bites) or spread to the circulatory system from infections in other body sites. For example, microorganisms causing pneumonia or renal infection may enter the local circulation of the lung or kidney and spread from there throughout the circulatory network.

If microbes in the bloodstream are not quickly eliminated, they can spread rapidly throughout the body, leading to serious, even life-threatening infections. Various terms are used to describe conditions involving microbes in the circulatory system. The term **bacteremia** refers to bacteria in the blood. If bacteria are reproducing in the blood as they spread, this condition is called **septicemia**. The presence of viruses in the blood is called **viremia**. Microbial toxins can also be spread through the circulatory system, causing a condition termed **toxemia**.

Microbes and microbial toxins in the blood can trigger an inflammatory response so severe that the inflammation damages host tissues and organs more than the infection itself. This counterproductive immune response is called **systemic inflammatory response syndrome (SIRS)**, and it can lead to the life-threatening condition known as **sepsis**. Sepsis is characterized by the production of excess cytokines that leads to classic signs of inflammation such as fever, vasodilation, and edema (see [Inflammation and Fever](#)). In a patient with sepsis, the inflammatory response becomes dysregulated and disproportionate to the threat of infection. Critical organs such as the heart, lungs, liver, and kidneys become dysfunctional, resulting in increased heart and respiratory rates, and disorientation. If not treated promptly and effectively, patients with sepsis can go into shock and die.

Certain infections can cause inflammation in the heart and blood vessels. Inflammation of the endocardium, the inner lining of the heart, is called **endocarditis** and can result in damage to the heart valves severe enough to require surgical replacement. Inflammation of the pericardium, the sac surrounding the heart, is called **pericarditis**. The term **myocarditis** refers to the inflammation of the heart's muscle tissue. Pericarditis and myocarditis can cause fluid to accumulate around the heart, resulting in congestive heart failure. Inflammation of blood vessels is called **vasculitis**. Although somewhat rare, vasculitis can cause blood vessels to become damaged and rupture; as blood is released, small red or purple spots called **petechiae** appear on the skin. If the damage of tissues or blood vessels is severe, it can result in reduced blood flow to the surrounding tissues. This condition is called **ischemia**, and it can be very serious. In severe cases, the affected tissues can die and become necrotic; these situations may require surgical debridement or amputation.

Note:

- Why does the circulatory system have no normal microbiota?
- Explain why the presence of microbes in the circulatory system can lead to serious consequences.

Infections of the Lymphatic System

Like the circulatory system, the lymphatic system does not have a normal microbiota, and the large numbers of immune cells typically eliminate transient microbes before they can establish an infection. Only microbes with an array of virulence factors are able to overcome these defenses and establish infection in the lymphatic system. However, when a localized infection begins to spread, the lymphatic system is often the first place the invading microbes can be detected.

Infections in the lymphatic system also trigger an inflammatory response. Inflammation of lymphatic vessels, called **lymphangitis**, can produce visible red streaks under the skin. Inflammation in the lymph nodes can cause them to swell. A swollen lymph node is referred to as a **bubo**, and the condition is referred to as **lymphadenitis**.

Key Concepts and Summary

- The **circulatory system** moves blood throughout the body and has no normal microbiota.
- The **lymphatic system** moves fluids from the interstitial spaces of tissues toward the circulatory system and filters the lymph. It also has no normal microbiota.
- The circulatory and lymphatic systems are home to many components of the host immune defenses.
- Infections of the circulatory system may occur after a break in the skin barrier or they may enter the bloodstream at the site of a localized infection. Pathogens or toxins in the bloodstream can spread rapidly throughout the body and can provoke systemic and sometimes fatal inflammatory responses such as **SIRS**, **sepsis**, and **endocarditis**.
- Infections of the lymphatic system can cause **lymphangitis** and **lymphadenitis**.

Multiple Choice

Exercise:

Problem: Which term refers to an inflammation of the blood vessels?

- A. lymphangitis
- B. endocarditis
- C. pericarditis
- D. vasculitis

Solution:

D

Exercise:

Problem:

Which of the following is located in the interstitial spaces within tissues and releases nutrients, immune factors, and oxygen to those tissues?

- A. lymphatics
- B. arterioles
- C. capillaries
- D. veins

Solution:

C

Exercise:

Problem:

Which of these conditions results in the formation of a bubo?

- A. lymphangitis
- B. lymphadenitis
- C. ischemia

D. vasculitis

Solution:

B

Exercise:

Problem:

Which of the following is where are most microbes filtered out of the fluids that accumulate in the body tissues?

- A. spleen
- B. lymph nodes
- C. pericardium
- D. blood capillaries

Solution:

B

Fill in the Blank

Exercise:

Problem:

Vasculitis can cause blood to leak from damaged vessels, forming purple spots called _____.

Solution:

petechiae

Exercise:

Problem: The lymph reenters the vascular circulation at _____.

Solution:

the subclavian veins

Short Answer

Exercise:

Problem:

How do lymph nodes help to maintain a microbial-free circulatory and lymphatic system?

Critical Thinking

Exercise:

Problem:

What term refers to the red streaks seen on this patient's skin? What is likely causing this condition?



(credit: modification of work by Centers for Disease Control and Prevention)

Exercise:

Problem:

Why would septicemia be considered a more serious condition than bacteremia?

Bacterial Infections of the Circulatory and Lymphatic Systems

LEARNING OBJECTIVES

- Identify and compare bacteria that most commonly cause infections of the circulatory and lymphatic systems
- Compare the major characteristics of specific bacterial diseases affecting the circulatory and lymphatic systems

Bacteria can enter the circulatory and lymphatic systems through acute infections or breaches of the skin barrier or mucosa. Breaches may occur through fairly common occurrences, such as insect bites or small wounds. Even the act of tooth brushing, which can cause small ruptures in the gums, may introduce bacteria into the circulatory system. In most cases, the bacteremia that results from such common exposures is transient and remains below the threshold of detection. In severe cases, bacteremia can lead to septicemia with dangerous complications such as toxemia, sepsis, and septic shock. In these situations, it is often the immune response to the infection that results in the clinical signs and symptoms rather than the microbes themselves.

Bacterial Sepsis, Septic and Toxic Shock

At low concentrations, pro-inflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor- α (TNF- α) play important roles in the host's immune defenses. When they circulate systemically in larger amounts, however, the resulting immune response can be life threatening. IL-1 induces vasodilation (widening of blood vessels) and reduces the tight junctions between vascular endothelial cells, leading to widespread edema.

As fluids move out of circulation into tissues, blood pressure begins to drop. If left unchecked, the blood pressure can fall below the level necessary to maintain proper kidney and respiratory functions, a condition known as **septic shock**. In addition, the excessive release of cytokines during the inflammatory response can lead to the formation of blood clots. The loss of blood pressure and occurrence of blood clots can result in multiple organ failure and death.

Bacteria are the most common pathogens associated with the development of sepsis, and septic shock.^[footnote] The most common infection associated with sepsis is bacterial pneumonia (see [Bacterial Infections of the Respiratory Tract](#)), accounting for about half of all cases, followed by intra-abdominal infections ([Bacterial Infections of the Gastrointestinal Tract](#)) and urinary tract infections ([Bacterial Infections of the Urinary System](#)).^[footnote] Infections associated with superficial wounds, animal bites, and indwelling catheters may also lead to sepsis and septic shock.

S.P. LaRosa. “Sepsis.” 2010.

<http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/infectious-disease/sepsis/>.

D.C. Angus, T. Van der Poll. “Severe Sepsis and Septic Shock.” *New England Journal of Medicine* 369, no. 9 (2013):840–851.

These initially minor, localized infections can be caused by a wide range of different bacteria, including *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Pasteurella*, *Acinetobacter*, and members of the Enterobacteriaceae. However, if left untreated, infections by these gram-positive and gram-negative pathogens can potentially progress to sepsis, shock, and death.

Toxic Shock Syndrome and Streptococcal Toxic Shock-Like Syndrome

Toxemia associated with infections caused by *Staphylococcus aureus* can cause staphylococcal **toxic shock syndrome (TSS)**. Some strains of *S. aureus* produce a superantigen called toxic shock syndrome toxin-1 (TSST-1). TSS may occur as a complication of other localized or systemic infections such as pneumonia, osteomyelitis, sinusitis, and skin wounds (surgical, traumatic, or burns). Those at highest risk for staphylococcal TSS

are women with preexisting *S. aureus* colonization of the vagina who leave tampons, contraceptive sponges, diaphragms, or other devices in the vagina for longer than the recommended time.

Staphylococcal TSS is characterized by sudden onset of vomiting, diarrhea, myalgia, body temperature higher than 38.9 °C (102.0 °F), and rapid-onset hypotension with a systolic blood pressure less than 90 mm Hg for adults; a diffuse erythematous rash that leads to peeling and shedding skin 1 to 2 weeks after onset; and additional involvement of three or more organ systems.^[footnote] The mortality rate associated with staphylococcal TSS is less than 3% of cases.

Centers for Disease Control and Prevention. “Toxic Shock Syndrome (Other Than Streptococcal) (TSS) 2011 Case Definition.”

<https://wwwn.cdc.gov/nndss/conditions/toxic-shock-syndrome-other-than-streptococcal/case-definition/2011/>. Accessed July 25, 2016.

Diagnosis of staphylococcal TSS is based on clinical signs, symptoms, serologic tests to confirm bacterial species, and the detection of toxin production from staphylococcal isolates. Cultures of skin and blood are often negative; less than 5% are positive in cases of staphylococcal TSS. Treatment for staphylococcal TSS includes decontamination, debridement, vasopressors to elevate blood pressure, and antibiotic therapy with clindamycin plus vancomycin or daptomycin pending susceptibility results.

A syndrome with signs and symptoms similar to staphylococcal TSS can be caused by *Streptococcus pyogenes*. This condition, called **streptococcal toxic shock-like syndrome (STSS)**, is characterized by more severe pathophysiology than staphylococcal TSS,^[footnote] with about 50% of patients developing *S. pyogenes* bacteremia and necrotizing fasciitis. In contrast to staphylococcal TSS, STSS is more likely to cause acute respiratory distress syndrome (ARDS), a rapidly progressive disease characterized by fluid accumulation in the lungs that inhibits breathing and causes hypoxemia (low oxygen levels in the blood). STSS is associated with a higher mortality rate (20%–60%), even with aggressive therapy. STSS usually develops in patients with a streptococcal soft-tissue infection such as bacterial cellulitis, necrotizing fasciitis, pyomyositis (pus formation

in muscle caused by infection), a recent influenza A infection, or chickenpox.

Centers for Disease Control and Prevention. “Streptococcal Toxic Shock Syndrome (STSS) (*Streptococcus pyogenes*) 2010 Case Definition.”

<https://wwwn.cdc.gov/nndss/conditions/streptococcal-toxic-shock-syndrome/case-definition/2010/>. Accessed July 25, 2016.

Note:

- How can large amounts of pro-inflammatory cytokines lead to septic shock?

Note:

Part 2

Despite oxacillin therapy, Barbara’s condition continued to worsen over the next several days. Her fever increased to 40.1 °C (104.2 °F) and she began to experience chills, rapid breathing, and confusion. Her doctor suspected bacteremia by a drug-resistant bacterium and admitted Barbara to the hospital. Cultures of the surgical site and blood revealed *Staphylococcus aureus*. Antibiotic susceptibility testing confirmed that the isolate was methicillin-resistant *S. aureus* (MRSA). In response, Barbara’s doctor changed her antibiotic therapy to vancomycin and arranged to have the port and venous catheter removed.

- Why did Barbara’s infection not respond to oxacillin therapy?
- Why did the physician have the port and catheter removed?
- Based on the signs and symptoms described, what are some possible diagnoses for Barbara’s condition?

Jump to the [next](#) Clinical Focus feature box. Go back to the [previous](#) Clinical Focus feature box.

Puerperal Sepsis

A type of sepsis called **puerperal sepsis**, also known as puerperal infection, puerperal fever, or childbed fever, is a nosocomial infection associated with the period of puerperium—the time following childbirth during which the mother’s reproductive system returns to a nonpregnant state. Such infections may originate in the genital tract, breast, urinary tract, or a surgical wound. Initially the infection may be limited to the uterus or other local site of infection, but it can quickly spread, resulting in peritonitis, septicemia, and death. Before the 19th century work of Ignaz Semmelweis and the widespread acceptance of germ theory (see [Modern Foundations of Cell Theory](#)), puerperal sepsis was a major cause of mortality among new mothers in the first few days following childbirth.

Puerperal sepsis is often associated with *Streptococcus pyogenes*, but numerous other bacteria can also be responsible. Examples include gram-positive bacterial (e.g. *Streptococcus* spp., *Staphylococcus* spp., and *Enterococcus* spp.), gram-negative bacteria (e.g. *Chlamydia* spp., *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp.), as well as anaerobes such as *Peptostreptococcus* spp., *Bacteroides* spp., and *Clostridium* spp. In cases caused by *S. pyogenes*, the bacteria attach to host tissues using M protein and produce a carbohydrate capsule to avoid phagocytosis. *S. pyogenes* also produces a variety of exotoxins, like streptococcal pyrogenic exotoxins A and B, that are associated with virulence and may function as superantigens.

Diagnosis of puerperal fever is based on the timing and extent of fever and isolation, and identification of the etiologic agent in blood, wound, or urine specimens. Because there are numerous possible causes, antimicrobial susceptibility testing must be used to determine the best antibiotic for treatment. Nosocomial incidence of puerperal fever can be greatly reduced through the use of antiseptics during delivery and strict adherence to handwashing protocols by doctors, midwives, and nurses.

Infectious Arthritis

Also called **septic arthritis**, **infectious arthritis** can be either an acute or a chronic condition. Infectious arthritis is characterized by inflammation of joint tissues and is most often caused by bacterial pathogens. Most cases of acute infectious arthritis are secondary to bacteremia, with a rapid onset of moderate to severe joint pain and swelling that limits the motion of the affected joint. In adults and young children, the infective pathogen is most often introduced directly through injury, such as a wound or a surgical site, and brought to the joint through the circulatory system. Acute infections may also occur after joint replacement surgery. Acute infectious arthritis often occurs in patients with an immune system impaired by other viral and bacterial infections. *S. aureus* is the most common cause of acute septic arthritis in the general population of adults and young children. *Neisseria gonorrhoeae* is an important cause of acute infectious arthritis in sexually active individuals.

Chronic infectious arthritis is responsible for 5% of all infectious arthritis cases and is more likely to occur in patients with other illnesses or conditions. Patients at risk include those who have an HIV infection, a bacterial or fungal infection, prosthetic joints, rheumatoid arthritis (RA), or who are undergoing immunosuppressive chemotherapy. Onset is often in a single joint; there may be little or no pain, aching pain that may be mild, gradual swelling, mild warmth, and minimal or no redness of the joint area.

Diagnosis of infectious arthritis requires the aspiration of a small quantity of synovial fluid from the afflicted joint. Direct microscopic evaluation, culture, antimicrobial susceptibility testing, and polymerase chain reaction (PCR) analyses of the synovial fluid are used to identify the potential pathogen. Typical treatment includes administration of appropriate antimicrobial drugs based on antimicrobial susceptibility testing. For nondrug-resistant bacterial strains, β -lactams such as oxacillin and cefazolin are often prescribed for staphylococcal infections. Third-generation cephalosporins (e.g., ceftriaxone) are used for increasingly prevalent β -lactam-resistant *Neisseria* infections. Infections by *Mycobacterium* spp. or fungi are treated with appropriate long-term antimicrobial therapy. Even with treatment, the prognosis is often poor for those infected. About 40% of patients with nongonococcal infectious arthritis will suffer permanent joint

damage and mortality rates range from 5% to 20%.[\[footnote\]](#) Mortality rates are higher among the elderly.[\[footnote\]](#)

M.E. Shirtliff, Mader JT. “Acute Septic Arthritis.” *Clinical Microbiology Reviews* 15 no. 4 (2002):527–544.

J.R. Maneiro et al. “Predictors of Treatment Failure and Mortality in Native Septic Arthritis.” *Clinical Rheumatology* 34, no. 11 (2015):1961–1967.

Osteomyelitis

Osteomyelitis is an inflammation of bone tissues most commonly caused by infection. These infections can either be acute or chronic and can involve a variety of different bacteria. The most common causative agent of **osteomyelitis** is *S. aureus*. However, *M. tuberculosis*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *S. agalactiae*, species in the Enterobacteriaceae, and other microorganisms can also cause osteomyelitis, depending on which bones are involved. In adults, bacteria usually gain direct access to the bone tissues through trauma or a surgical procedure involving prosthetic joints. In children, the bacteria are often introduced from the bloodstream, possibly spreading from focal infections. The long bones, such as the femur, are more commonly affected in children because of the more extensive vascularization of bones in the young.[\[footnote\]](#)

M. Vazquez. “Osteomyelitis in Children.” *Current Opinion in Pediatrics* 14, no. 1 (2002):112–115.

The signs and symptoms of osteomyelitis include fever, localized pain, swelling due to edema, and ulcers in soft tissues near the site of infection. The resulting inflammation can lead to tissue damage and bone loss. In addition, the infection may spread to joints, resulting in infectious arthritis, or disseminate into the blood, resulting in sepsis and thrombosis (formation of blood clots). Like septic arthritis, osteomyelitis is usually diagnosed using a combination of radiography, imaging, and identification of bacteria from blood cultures, or from bone cultures if blood cultures are negative. Parenteral antibiotic therapy is typically used to treat osteomyelitis. Because of the number of different possible etiologic agents, however, a variety of drugs might be used. Broad-spectrum antibacterial drugs such as nafcillin, oxacillin, or cephalosporin are typically prescribed for acute osteomyelitis, and ampicillin and piperacillin/tazobactam for chronic

osteomyelitis. In cases of antibiotic resistance, vancomycin treatment is sometimes required to control the infection. In serious cases, surgery to remove the site of infection may be required. Other forms of treatment include hyperbaric oxygen therapy (see [Using Physical Methods to Control Microorganisms](#)) and implantation of antibiotic beads or pumps.

Note:

- What bacterium the most common cause of both septic arthritis and osteomyelitis?

Rheumatic Fever

Infections with *S. pyogenes* have a variety of manifestations and complications generally called sequelae. As mentioned, the bacterium can cause suppurative infections like puerperal fever. However, this microbe can also cause nonsuppurative sequelae in the form of acute **rheumatic fever** (ARF), which can lead to rheumatic heart disease, thus impacting the circulatory system. Rheumatic fever occurs primarily in children a minimum of 2–3 weeks after an episode of untreated or inadequately treated pharyngitis (see [Bacterial Infections of the Respiratory Tract](#)). At one time, rheumatic fever was a major killer of children in the US; today, however, it is rare in the US because of early diagnosis and treatment of streptococcal pharyngitis with antibiotics. In parts of the world where diagnosis and treatment are not readily available, acute rheumatic fever and rheumatic heart disease are still major causes of mortality in children.[\[footnote\]](#)

A. Beaudoin et al. “Acute Rheumatic Fever and Rheumatic Heart Disease Among Children—American Samoa, 2011–2012.” *Morbidity and Mortality Weekly Report* 64 no. 20 (2015):555–558.

Rheumatic fever is characterized by a variety of diagnostic signs and symptoms caused by nonsuppurative, immune-mediated damage resulting from a cross-reaction between patient antibodies to bacterial surface

proteins and similar proteins found on cardiac, neuronal, and synovial tissues. Damage to the nervous tissue or joints, which leads to joint pain and swelling, is reversible. However, damage to heart valves can be irreversible and is worsened by repeated episodes of acute rheumatic fever, particularly during the first 3–5 years after the first rheumatic fever attack. The inflammation of the heart valves caused by cross-reacting antibodies leads to scarring and stiffness of the valve leaflets. This, in turn, produces a characteristic heart murmur. Patients who have previously developed rheumatic fever and who subsequently develop recurrent pharyngitis due to *S. pyogenes* are at high risk for a recurrent attacks of rheumatic fever.

The American Heart Association recommends[\[footnote\]](#) a treatment regimen consisting of benzathine benzylpenicillin every 3 or 4 weeks, depending on the patient's risk for reinfection. Additional prophylactic antibiotic treatment may be recommended depending on the age of the patient and risk for reinfection.

M.A. Gerber et al. "Prevention of Rheumatic Fever and Diagnosis and Treatment of Acute Streptococcal Pharyngitis: A Scientific Statement From the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research: Endorsed by the American Academy of Pediatrics." *Circulation* 119, no. 11 (2009):1541–1551.

Bacterial Endocarditis and Pericarditis

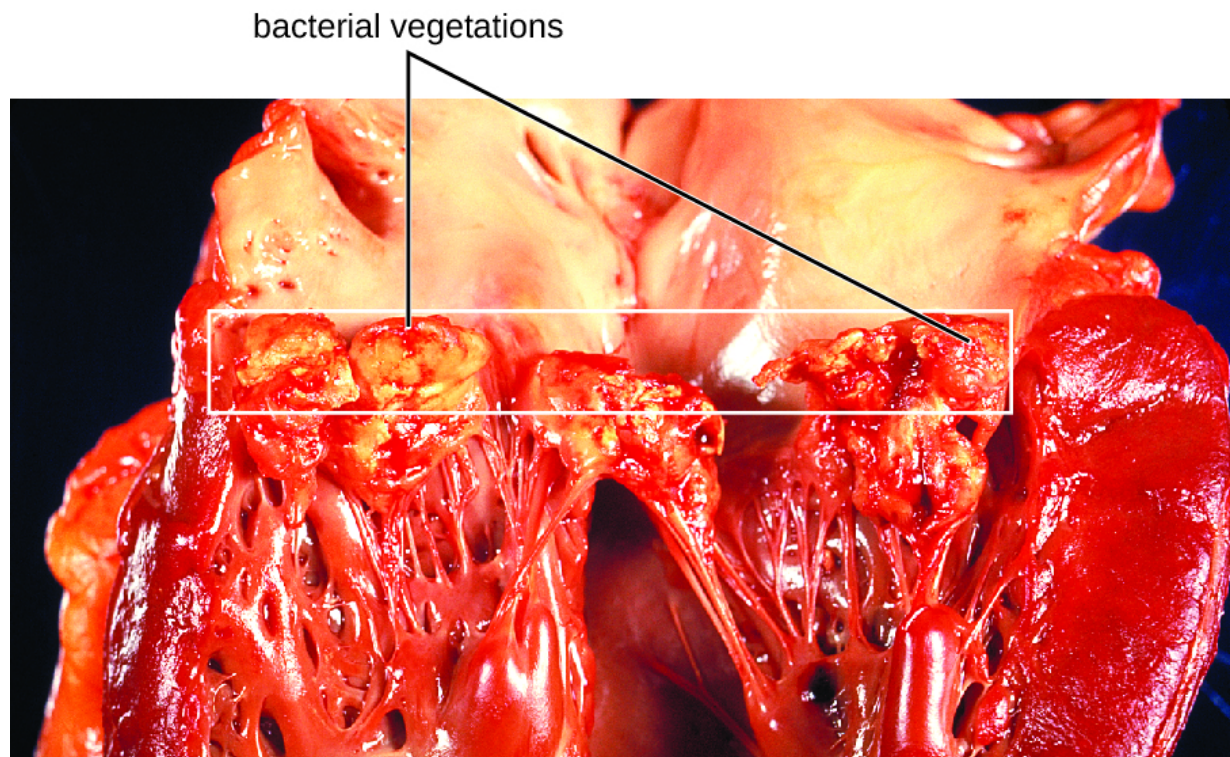
The endocardium is a tissue layer that lines the muscles and valves of the heart. This tissue can become infected by a variety of bacteria, including gram-positive cocci such as *Staphylococcus aureus*, viridans streptococci, and *Enterococcus faecalis*, and the gram-negative so-called HACEK bacilli: *Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*. The resulting inflammation is called endocarditis, which can be described as either acute or subacute. Causative agents typically enter the bloodstream during accidental or intentional breaches in the normal barrier defenses (e.g., dental procedures, body piercings, catheterization, wounds).

Individuals with preexisting heart damage, prosthetic valves and other cardiac devices, and those with a history of rheumatic fever have a higher risk for endocarditis. This disease can rapidly destroy the heart valves and, if untreated, lead to death in just a few days.

In **subacute bacterial endocarditis**, heart valve damage occurs slowly over a period of months. During this time, blood clots form in the heart, and these protect the bacteria from phagocytes. These patches of tissue-associated bacteria are called vegetations. The resulting damage to the heart, in part resulting from the immune response causing fibrosis of heart valves, can necessitate heart valve replacement ([\[link\]](#)). Outward signs of subacute endocarditis may include a fever.

Diagnosis of infective endocarditis is determined using the combination of blood cultures, echocardiogram, and clinical symptoms. In both acute and subacute endocarditis, treatment typically involves relatively high doses of intravenous antibiotics as determined by antimicrobial susceptibility testing. Acute endocarditis is often treated with a combination of ampicillin, nafcillin, and gentamicin for synergistic coverage of *Staphylococcus* spp. and *Streptococcus* spp. Prosthetic-valve endocarditis is often treated with a combination of vancomycin, rifampin, and gentamicin. Rifampin is necessary to treat individuals with infection of prosthetic valves or other foreign bodies because rifampin can penetrate the biofilm of most of the pathogens that infect these devices.

Staphylococcus spp. and *Streptococcus* spp. can also infect and cause inflammation in the tissues surrounding the heart, a condition called acute pericarditis. Pericarditis is marked by chest pain, difficulty breathing, and a dry cough. In most cases, pericarditis is self-limiting and clinical intervention is not necessary. Diagnosis is made with the aid of a chest radiograph, electrocardiogram, echocardiogram, aspirate of pericardial fluid, or biopsy of pericardium. Antibacterial medications may be prescribed for infections associated with pericarditis; however, pericarditis can also be caused other pathogens, including viruses (e.g., echovirus, influenza virus), fungi (e.g., *Histoplasma* spp., *Coccidioides* spp.), and eukaryotic parasites (e.g., *Toxoplasma* spp.).



The heart of an individual who had subacute bacterial endocarditis of the mitral valve. Bacterial vegetations are visible on the valve tissues.
(credit: modification of work by Centers for Disease Control and Prevention)

Note:

- Compare acute and subacute bacterial endocarditis.

Gas Gangrene

Traumatic injuries or certain medical conditions, such as diabetes, can cause damage to blood vessels that interrupts blood flow to a region of the body.

When blood flow is interrupted, tissues begin to die, creating an anaerobic environment in which anaerobic bacteria can thrive. This condition is called ischemia. Endospores of the anaerobic bacterium *Clostridium perfringens* (along with a number of other *Clostridium* spp. from the gut) can readily germinate in ischemic tissues and colonize the anaerobic tissues.

The resulting infection, called **gas gangrene**, is characterized by rapidly spreading myonecrosis (death of muscle tissue). The patient experiences a sudden onset of excruciating pain at the infection site and the rapid development of a foul-smelling wound containing gas bubbles and a thin, yellowish discharge tinged with a small amount of blood. As the infection progresses, edema and cutaneous blisters containing bluish-purple fluid form. The infected tissue becomes liquefied and begins sloughing off. The margin between necrotic and healthy tissue often advances several inches per hour even with antibiotic therapy. Septic shock and organ failure frequently accompany gas gangrene; when patients develop sepsis, the mortality rate is greater than 50%.

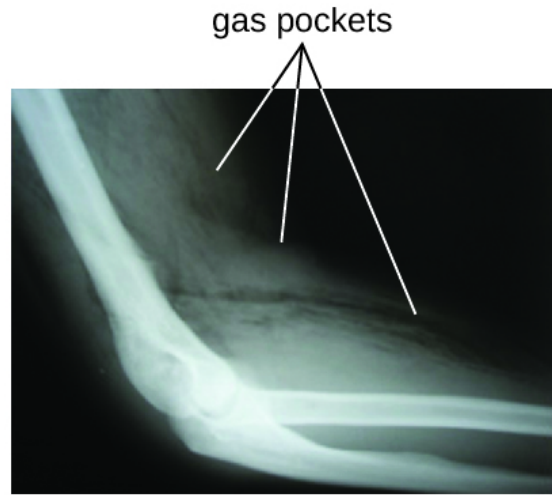
α -Toxin and theta (θ) toxin are the major virulence factors of *C. perfringens* implicated in gas gangrene. α -Toxin is a lipase responsible for breaking down cell membranes; it also causes the formation of thrombi (blood clots) in blood vessels, contributing to the spread of ischemia. θ -Toxin forms pores in the patient's cell membranes, causing cell lysis. The gas associated with gas gangrene is produced by *Clostridium*'s fermentation of butyric acid, which produces hydrogen and carbon dioxide that are released as the bacteria multiply, forming pockets of gas in tissues ([\[link\]](#)).

Gas gangrene is initially diagnosed based on the presence of the clinical signs and symptoms described earlier in this section. Diagnosis can be confirmed through Gram stain and anaerobic cultivation of wound exudate (drainage) and tissue samples on blood agar. Treatment typically involves surgical debridement of any necrotic tissue; advanced cases may require amputation. Surgeons may also use vacuum-assisted closure (VAC), a surgical technique in which vacuum-assisted drainage is used to remove blood or serous fluid from a wound or surgical site to speed recovery. The most common antibiotic treatments include penicillin G and clindamycin.

Some cases are also treated with hyperbaric oxygen therapy because *Clostridium* spp. are incapable of surviving in oxygen-rich environments.



(a)



(b)

(a) In this image of a patient with gas gangrene, note the bluish-purple discoloration around the bicep and the irregular margin of the discolored tissue indicating the spread of infection. (b) A radiograph of the arm shows a darkening in the tissue, which indicates the presence of gas. (credit a, b: modification of work by Aggelidakis J, Lasithiotakis K, Topalidou A, Koutroumpas J, Kouvidis G, and Katonis P)

Tularemia

Infection with the gram-negative bacterium *Francisella tularensis* causes **tularemia** (or rabbit fever), a zoonotic infection in humans. *F. tularensis* is a facultative intracellular parasite that primarily causes illness in rabbits, although a wide variety of domesticated animals are also susceptible to infection. Humans can be infected through ingestion of contaminated meat or, more typically, handling of infected animal tissues (e.g., skinning an infected rabbit). Tularemia can also be transmitted by the bites of infected

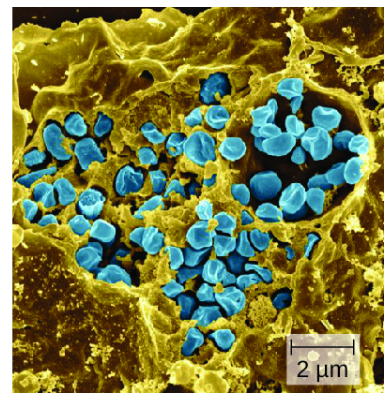
arthropods, including the dog tick (*Dermacentor variabilis*), the lone star tick (*Amblyomma americanum*), the wood tick (*Dermacentor andersoni*), and deer flies (*Chrysops* spp.). Although the disease is not directly communicable between humans, exposure to aerosols of *F. tularensis* can result in life-threatening infections. *F. tularensis* is highly contagious, with an infectious dose of as few as 10 bacterial cells. In addition, pulmonary infections have a 30%–60% fatality rate if untreated.[\[footnote\]](#) For these reasons, *F. tularensis* is currently classified and must be handled as a biosafety level-3 (BSL-3) organism and as a potential biological warfare agent.

World Health Organization. “WHO Guidelines on Tularaemia.” 2007.
<http://www.cdc.gov/tularemia/resources/whotularemiamanual.pdf>.
Accessed July 26, 2016.

Following introduction through a break in the skin, the bacteria initially move to the lymph nodes, where they are ingested by phagocytes. After escaping from the phagosome, the bacteria grow and multiply intracellularly in the cytoplasm of phagocytes. They can later become disseminated through the blood to other organs such as the liver, lungs, and spleen, where they produce masses of tissue called granulomas ([\[link\]](#)). After an incubation period of about 3 days, skin lesions develop at the site of infection. Other signs and symptoms include fever, chills, headache, and swollen and painful lymph nodes.



(a)



(b)

(a) A skin lesion appears at the site of infection on the hand of an

individual infected with *Francisella tularensis*. (b) A scanning electron micrograph shows the coccobacilli cells (blue) of *F. tularensis*. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by NIAID)

A direct diagnosis of tularemia is challenging because it is so contagious. Once a presumptive diagnosis of tularemia is made, special handling is required to collect and process patients' specimens to prevent the infection of health-care workers. Specimens suspected of containing *F. tularensis* can only be handled by BSL-2 or BSL-3 laboratories registered with the Federal Select Agent Program, and individuals handling the specimen must wear protective equipment and use a class II biological safety cabinet.

Tularemia is relatively rare in the US, and its signs and symptoms are similar to a variety of other infections that may need to be ruled out before a diagnosis can be made. Direct fluorescent-antibody (DFA) microscopic examination using antibodies specific for *F. tularensis* can rapidly confirm the presence of this pathogen. Culturing this microbe is difficult because of its requirement for the amino acid cysteine, which must be supplied as an extra nutrient in culturing media. Serological tests are available to detect an immune response against the bacterial pathogen. In patients with suspected infection, acute- and convalescent-phase serum samples are required to confirm an active infection. PCR-based tests can also be used for clinical identification of direct specimens from body fluids or tissues as well as cultured specimens. In most cases, diagnosis is based on clinical findings and likely incidents of exposure to the bacterium. The antibiotics streptomycin, gentamycin, doxycycline, and ciprofloxacin are effective in treating tularemia.

Brucellosis

Species in the genus *Brucella* are gram-negative facultative intracellular pathogens that appear as coccobacilli. Several species cause zoonotic infections in animals and humans, four of which have significant human pathogenicity: *B. abortus* from cattle and buffalo, *B. canis* from dogs, *B.*

suis from swine, and *B. melitensis* from goats, sheep, and camels. Infections by these pathogens are called brucellosis, also known as undulant fever, “Mediterranean fever,” or “Malta fever.” Vaccination of animals has made brucellosis a rare disease in the US, but it is still common in the Mediterranean, south and central Asia, Central and South America, and the Caribbean. Human infections are primarily associated with the ingestion of meat or unpasteurized dairy products from infected animals. Infection can also occur through inhalation of bacteria in aerosols when handling animal products, or through direct contact with skin wounds. In the US, most cases of brucellosis are found in individuals with extensive exposure to potentially infected animals (e.g., slaughterhouse workers, veterinarians).

Two important virulence factors produced by *Brucella* spp. are urease, which allows ingested bacteria to avoid destruction by stomach acid, and lipopolysaccharide (LPS), which allows the bacteria to survive within phagocytes. After gaining entry to tissues, the bacteria are phagocytized by host neutrophils and macrophages. The bacteria then escape from the phagosome and grow within the cytoplasm of the cell. Bacteria phagocytized by macrophages are disseminated throughout the body. This results in the formation of granulomas within many body sites, including bone, liver, spleen, lung, genitourinary tract, brain, heart, eye, and skin. Acute infections can result in undulant (relapsing) fever, but untreated infections develop into chronic disease that usually manifests as acute febrile illness (fever of 40–41 °C [104–105.8 °F]) with recurring flu-like signs and symptoms.

Brucella is only reliably found in the blood during the acute fever stage; it is difficult to diagnose by cultivation. In addition, *Brucella* is considered a BSL-3 pathogen and is hazardous to handle in the clinical laboratory without protective clothing and at least a class II biological safety cabinet. Agglutination tests are most often used for serodiagnosis. In addition, enzyme-linked immunosorbent assays (ELISAs) are available to determine exposure to the organism. The antibiotics doxycycline or ciprofloxacin are typically prescribed in combination with rifampin; gentamicin, streptomycin, and trimethoprim-sulfamethoxazole (TMP-SMZ) are also effective against *Brucella* infections and can be used if needed.

Note:

- Compare the pathogenesis of tularemia and brucellosis.

Cat-Scratch Disease

The zoonosis **cat-scratch disease (CSD)** (or cat-scratch fever) is a bacterial infection that can be introduced to the lymph nodes when a human is bitten or scratched by a cat. It is caused by the facultative intracellular gram-negative bacterium *Bartonella henselae*. Cats can become infected from flea feces containing *B. henselae* that they ingest while grooming. Humans become infected when flea feces or cat saliva (from claws or licking) containing *B. henselae* are introduced at the site of a bite or scratch. Once introduced into a wound, *B. henselae* infects red blood cells.

B. henselae invasion of red blood cells is facilitated by adhesins associated with outer membrane proteins and a secretion system that mediates transport of virulence factors into the host cell. Evidence of infection is indicated if a small nodule with pus forms in the location of the scratch 1 to 3 weeks after the initial injury. The bacteria then migrate to the nearest lymph nodes, where they cause swelling and pain. Signs and symptoms may also include fever, chills, and fatigue. Most infections are mild and tend to be self-limiting. However, immunocompromised patients may develop bacillary angiomatosis (BA), characterized by the proliferation of blood vessels, resulting in the formation of tumor-like masses in the skin and internal organs; or bacillary peliosis (BP), characterized by multiple cyst-like, blood-filled cavities in the liver and spleen. Most cases of CSD can be prevented by keeping cats free of fleas and promptly cleaning a cat scratch with soap and warm water.

The diagnosis of CSD is difficult because the bacterium does not grow readily in the laboratory. When necessary, immunofluorescence, serological tests, PCR, and gene sequencing can be performed to identify the bacterial species. Given the limited nature of these infections, antibiotics are not normally prescribed. For immunocompromised patients, rifampin,

azithromycin, ciprofloxacin, gentamicin (intramuscularly), or TMP-SMZ are generally the most effective options.

Rat-Bite Fever

The zoonotic infection **rat-bite fever** can be caused by two different gram-negative bacteria: *Streptobacillus moniliformis*, which is more common in North America, and *Spirillum minor*, which is more common in Asia. Because of modern sanitation efforts, rat bites are rare in the US. However, contact with fomites, food, or water contaminated by rat feces or body fluids can also cause infections. Signs and symptoms of rat-bite fever include fever, vomiting, myalgia (muscle pain), arthralgia (joint pain), and a maculopapular rash on the hands and feet. An ulcer may also form at the site of a bite, along with some swelling of nearby lymph nodes. In most cases, the infection is self-limiting. Little is known about the virulence factors that contribute to these signs and symptoms of disease.

Cell culture, MALDI-TOF mass spectrometry, PCR, or ELISA can be used in the identification of *Streptobacillus moniliformis*. The diagnosis *Spirillum minor* may be confirmed by direct microscopic observation of the pathogens in blood using Giemsa or Wright stains, or darkfield microscopy. Serological tests can be used to detect a host immune response to the pathogens after about 10 days. The most commonly used antibiotics to treat these infections are penicillin or doxycycline.

Plague

The gram-negative bacillus *Yersinia pestis* causes the zoonotic infection **plague**. This bacterium causes acute febrile disease in animals, usually rodents or other small mammals, and humans. The disease is associated with a high mortality rate if left untreated. Historically, *Y. pestis* has been responsible for several devastating pandemics, resulting in millions of deaths (see [Micro Connections: The History of the Plague](#)). There are three forms of plague: **bubonic plague** (the most common form, accounting for about 80% of cases), **pneumonic plague**, and **septicemic plague**. These forms are differentiated by the mode of transmission and the initial site of

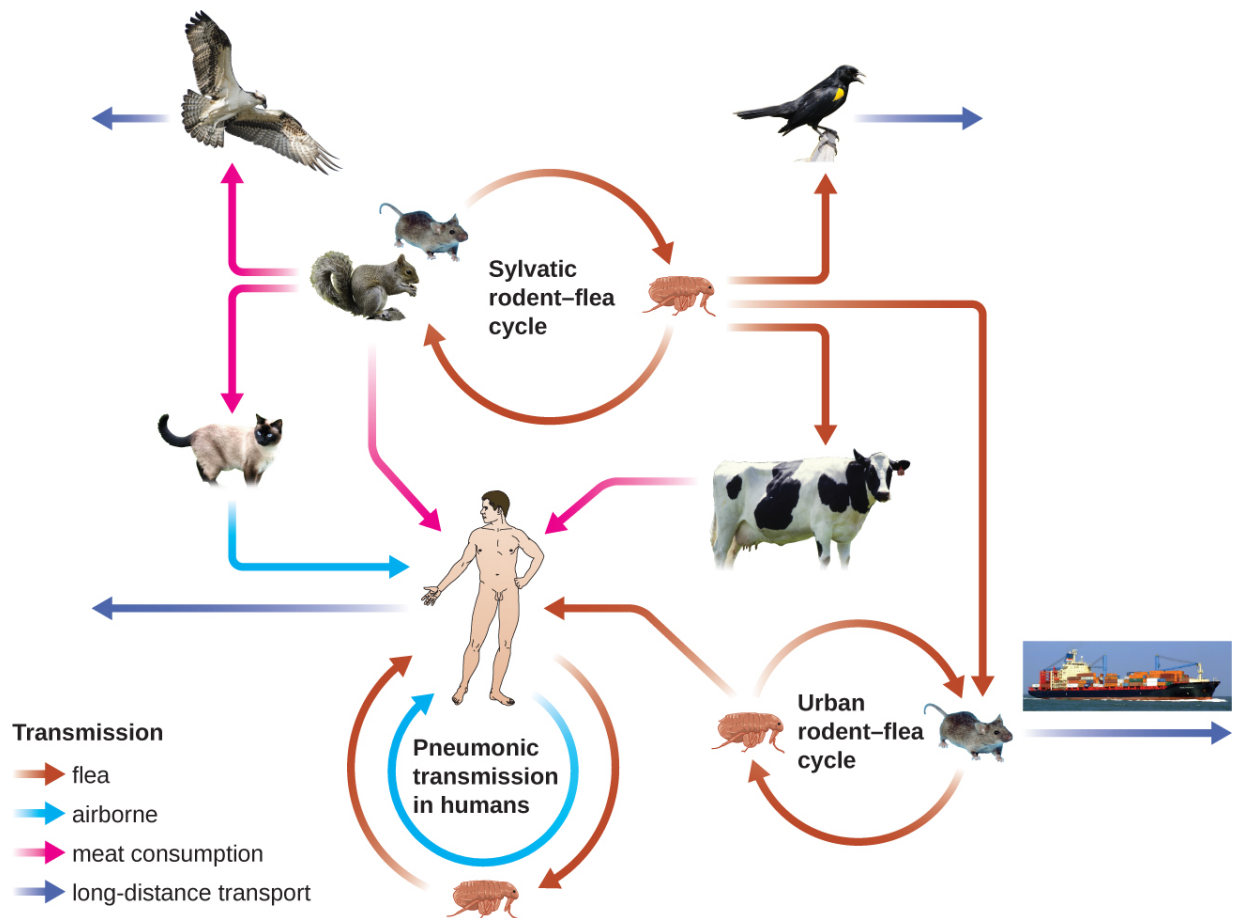
infection. [\[link\]](#) illustrates these various modes of transmission and infection between animals and humans.

In bubonic plague, *Y. pestis* is transferred by the bite of infected fleas. Since most flea bites occur on the legs and ankles, *Y. pestis* is often introduced into the tissues and blood circulation in the lower extremities. After a 2- to 6-day incubation period, patients experience an abrupt onset fever (39.5–41 °C [103.1–105.8 °F]), headache, hypotension, and chills. The pathogen localizes in lymph nodes, where it causes inflammation, swelling, and hemorrhaging that results in purple buboes ([\[link\]](#)). Buboes often form in lymph nodes of the groin first because these are the nodes associated with the lower limbs; eventually, through circulation in the blood and lymph, lymph nodes throughout the body become infected and form buboes. The average mortality rate for bubonic plague is about 55% if untreated and about 10% with antibiotic treatment.

Septicemic plague occurs when *Y. pestis* is directly introduced into the bloodstream through a cut or wound and circulates through the body. The incubation period for septicemic plague is 1 to 3 days, after which patients develop fever, chills, extreme weakness, abdominal pain, and shock. Disseminated intravascular coagulation (DIC) can also occur, resulting in the formation of thrombi that obstruct blood vessels and promote ischemia and necrosis in surrounding tissues ([\[link\]](#)). Necrosis occurs most commonly in extremities such as fingers and toes, which become blackened. Septicemic plague can quickly lead to death, with a mortality rate near 100% when it is untreated. Even with antibiotic treatment, the mortality rate is about 50%.

Pneumonic plague occurs when *Y. pestis* causes an infection of the lungs. This can occur through inhalation of aerosolized droplets from an infected individual or when the infection spreads to the lungs from elsewhere in the body in patients with bubonic or septicemic plague. After an incubation period of 1 to 3 days, signs and symptoms include fever, headache, weakness, and a rapidly developing pneumonia with shortness of breath, chest pain, and cough producing bloody or watery mucus. The pneumonia may result in rapid respiratory failure and shock. Pneumonic plague is the only form of plague that can be spread from person to person by infectious

aerosol droplet. If untreated, the mortality rate is near 100%; with antibiotic treatment, the mortality rate is about 50%.



Yersinia pestis, the causative agent of plague, has numerous modes of transmission. The modes are divided into two ecological classes: urban and sylvatic (i.e., forest or rural). The urban cycle primarily involves transmission from infected urban mammals (rats) to humans by flea vectors (brown arrows). The disease may travel between urban centers (purple arrow) if infected rats find their way onto ships or trains. The sylvatic cycle involves mammals more common in nonurban environments. Sylvatic birds and mammals (including humans) may become infected after eating infected mammals (pink arrows) or by flea vectors. Pneumonic transmission occurs between humans or between humans and infected animals through the inhalation of *Y. pestis* in aerosols. (credit “diagram”: modification of work by Stenseth)

NC, Atshabar BB, Begon M, Belmain SR, Bertherat E, Carniel E, Gage KL, Leirs H, and Rahalison L; credit “cat”: modification of work by “KaCey97078”/Flickr)



(a)



(b)

(a) *Yersinia pestis* infection can cause inflamed and swollen lymph nodes (buboes), like these in the groin of an infected patient. (b)

Septicemic plague caused necrotic toes in this patient. Vascular damage at the extremities causes ischemia and tissue death. (credit a: modification of work by American Society for Microbiology; credit b: modification of work by Centers for Disease Control and Prevention)

The high mortality rate for the plague is, in part, a consequence of it being unusually well equipped with virulence factors. To date, there are at least 15 different major virulence factors that have been identified from *Y. pestis* and, of these, eight are involved with adherence to host cells. In addition,

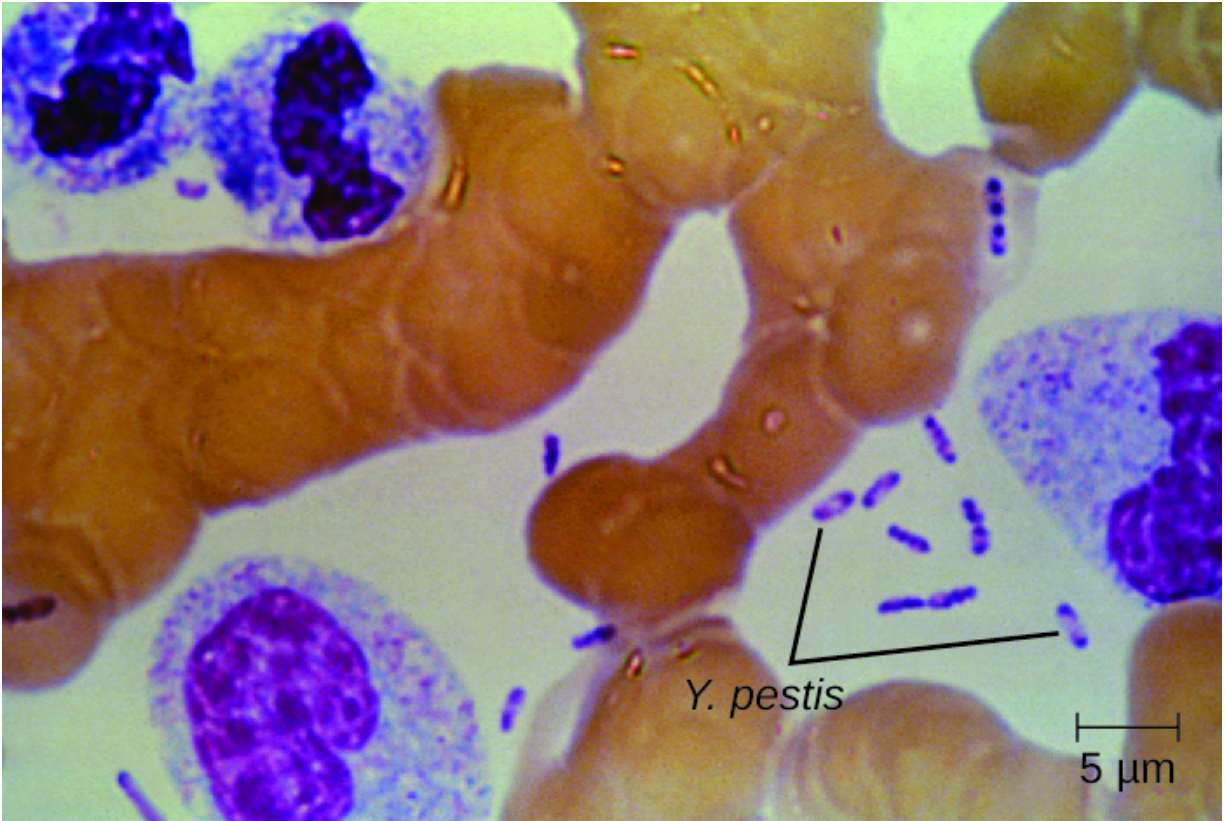
the F1 component of the *Y. pestis* capsule is a virulence factor that allows the bacterium to avoid phagocytosis. F1 is produced in large quantities during mammalian infection and is the most immunogenic component.

[[footnote](#)] Successful use of virulence factors allows the bacilli to disseminate from the area of the bite to regional lymph nodes and eventually the entire blood and lymphatic systems.

MOH Key Laboratory of Systems Biology of Pathogens. “Virulence Factors of Pathogenic Bacteria, *Yersinia*.” <http://www.mgc.ac.cn/cgi-bin/VFs/genus.cgi?Genus=Yersinia>. Accessed September 9, 2016.

Culturing and direct microscopic examination of a sample of fluid from a bubo, blood, or sputum is the best way to identify *Y. pestis* and confirm a presumptive diagnosis of plague. Specimens may be stained using either a Gram, Giemsa, Wright, or Wayson's staining technique ([link](#)). The bacteria show a characteristic bipolar staining pattern, resembling safety pins, that facilitates presumptive identification. Direct fluorescent antibody tests (rapid test of outer-membrane antigens) and serological tests like ELISA can be used to confirm the diagnosis. The confirmatory method for identifying *Y. pestis* isolates in the US is bacteriophage lysis.

Prompt antibiotic therapy can resolve most cases of bubonic plague, but septicemic and pneumonic plague are more difficult to treat because of their shorter incubation stages. Survival often depends on an early and accurate diagnosis and an appropriate choice of antibiotic therapy. In the US, the most common antibiotics used to treat patients with plague are gentamicin, fluoroquinolones, streptomycin, levofloxacin, ciprofloxacin, and doxycycline.



This Wright's stain of a blood sample from a patient with plague shows the characteristic “safety pin” appearance of *Yersinia pestis*.
(credit: modification of work by Centers for Disease Control and Prevention)

Note:

- Compare bubonic plague, septicemic plague, and pneumonic plague.

Note:

The History of the Plague

The first recorded pandemic of plague, the Justinian plague, occurred in the sixth century CE. It is thought to have originated in central Africa and spread to the Mediterranean through trade routes. At its peak, more than 5,000 people died per day in Constantinople alone. Ultimately, one-third of that city's population succumbed to plague.[\[footnote\]](#) The impact of this outbreak probably contributed to the later fall of Emperor Justinian.

Rosen, William. *Justinian's Flea: Plague, Empire, and the Birth of Europe*. Viking Adult; pg 3; ISBN 978-0-670-03855-8.

The second major pandemic, dubbed the Black Death, occurred during the 14th century. This time, the infections are thought to have originated somewhere in Asia before being transported to Europe by trade, soldiers, and war refugees. This outbreak killed an estimated one-quarter of the population of Europe (25 million, primarily in major cities). In addition, at least another 25 million are thought to have been killed in Asia and Africa.[\[footnote\]](#) This second pandemic, associated with strain *Yersinia pestis* biovar Medievalis, cycled for another 300 years in Europe and Great Britain, and was called the Great Plague in the 1660s.

Benedictow, Ole J. 2004. *The Black Death 1346-1353: The Complete History*. Woodbridge: Boydell Press.

The most recent pandemic occurred in the 1890s with *Yersinia pestis* biovar Orientalis. This outbreak originated in the Yunnan province of China and spread worldwide through trade. It is at this time that plague made its way to the US. The etiologic agent of plague was discovered by Alexandre Yersin (1863–1943) during this outbreak as well. The overall number of deaths was lower than in prior outbreaks, perhaps because of improved sanitation and medical support.[\[footnote\]](#) Most of the deaths attributed to this final pandemic occurred in India.

Centers for Disease Control and Prevention. "Plague: History." <http://www.cdc.gov/plague/history/>. Accessed September 15, 2016.

Note:

Visit this [link](#) to see a video describing how similar the genome of the Black Death bacterium is to today's strains of bubonic plague.

Zoonotic Febrile Diseases

A wide variety of zoonotic febrile diseases (diseases that cause fever) are caused by pathogenic bacteria that require arthropod vectors. These pathogens are either obligate intracellular species of *Anaplasma*, *Bartonella*, *Ehrlichia*, *Orientia*, and *Rickettsia*, or spirochetes in the genus *Borrelia*. Isolation and identification of pathogens in this group are best performed in BSL-3 laboratories because of the low infective dose associated with the diseases.

Anaplasmosis

The zoonotic tickborne disease **human granulocytic anaplasmosis (HGA)** is caused by the obligate intracellular pathogen *Anaplasma phagocytophilum*. HGA is endemic primarily in the central and northeastern US and in countries in Europe and Asia.

HGA is usually a mild febrile disease that causes flu-like symptoms in immunocompetent patients; however, symptoms are severe enough to require hospitalization in at least 50% of infections and, of those patients, less than 1% will die of HGA.[\[footnote\]](#) Small mammals such as white-footed mice, chipmunks, and voles have been identified as reservoirs of *A. phagocytophilum*, which is transmitted by the bite of an *Ixodes* tick. Five major virulence factors[\[footnote\]](#) have been reported in *Anaplasma*; three are adherence factors and two are factors that allow the pathogen to avoid the human immune response. Diagnostic approaches include locating intracellular microcolonies of *Anaplasma* through microscopic examination of neutrophils or eosinophils stained with Giemsa or Wright stain, PCR for detection of *A. phagocytophilum*, and serological tests to detect antibody titers against the pathogens. The primary antibiotic used for treatment is doxycycline.

J.S. Bakken et al. "Diagnosis and Management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever, Ehrlichioses, and Anaplasmosis—United States. A Practical Guide for Physicians and Other Health Care and Public Health Professionals." *MMWR Recommendations and Reports* 55 no. RR04 (2006):1–27.

MOH Key Laboratory of Systems Biology of Pathogens, “Virulence Factors of Pathogenic Bacteria, Anaplasma” 2016.
<http://www.mgc.ac.cn/cgi-bin/VFs/jsif/main.cgi>. Accessed July, 26, 2016.

Ehrlichiosis

Human monocytotropic ehrlichiosis (HME) is a zoonotic tickborne disease caused by the BSL-2, obligate intracellular pathogen *Ehrlichia chaffeensis*. Currently, the geographic distribution of HME is primarily the eastern half of the US, with a few cases reported in the West, which corresponds with the known geographic distribution of the primary vector, the lone star tick (*Amblyomma americanum*). Symptoms of HME are similar to the flu-like symptoms observed in anaplasmosis, but a rash is more common, with 60% of children and less than 30% of adults developing petechial, macula, and maculopapular rashes.[\[footnote\]](#) Virulence factors allow *E. chaffeensis* to adhere to and infect monocytes, forming intracellular microcolonies in monocytes that are diagnostic for the HME. Diagnosis of HME can be confirmed with PCR and serologic tests. The first-line treatment for adults and children of all ages with HME is doxycycline.

Centers for Disease Control and Prevention. “Ehrlichiosis, Symptoms, Diagnosis, and Treatment.” 2016.

<https://www.cdc.gov/ehrlichiosis/symptoms/index.html>. Accessed July 29, 2016.

Epidemic Typhus

The disease **epidemic typhus** is caused by *Rickettsia prowazekii* and is transmitted by body lice, *Pediculus humanus*. Flying squirrels are animal reservoirs of *R. prowazekii* in North America and can also be sources of lice capable of transmitting the pathogen. Epidemic typhus is characterized by a high fever and body aches that last for about 2 weeks. A rash develops on the abdomen and chest and radiates to the extremities. Severe cases can result in death from shock or damage to heart and brain tissues. Infected

humans are an important reservoir for this bacterium because *R. prowazekii* is the only *Rickettsia* that can establish a chronic carrier state in humans.

Epidemic typhus has played an important role in human history, causing large outbreaks with high mortality rates during times of war or adversity. During World War I, epidemic typhus killed more than 3 million people on the Eastern front.[\[footnote\]](#) With the advent of effective insecticides and improved personal hygiene, epidemic typhus is now quite rare in the US. In the developing world, however, epidemics can lead to mortality rates of up to 40% in the absence of treatment.[\[footnote\]](#) In recent years, most outbreaks have taken place in Burundi, Ethiopia, and Rwanda. For example, an outbreak in Burundi refugee camps in 1997 resulted in 45,000 illnesses in a population of about 760,000 people.[\[footnote\]](#)

Drali, R., Brouqui, P. and Raoult, D. “Typhus in World War I.” *Microbiology Today* 41 (2014) 2:58–61.

Centers for Disease Control and Prevention. *CDC Health Information for International Travel 2014: The Yellow Book*. Oxford University Press, 2013. <http://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/rickettsial-spotted-typhus-fevers-related-infections-anaplasmosis-ehrlichiosis>. Accessed July 26, 2016.

World Health Organization. “Typhus.” 1997. <http://www.who.int/mediacentre/factsheets/fs162/en/>. Accessed July 26, 2016.

A rapid diagnosis is difficult because of the similarity of the primary symptoms with those of many other diseases. Molecular and immunohistochemical diagnostic tests are the most useful methods for establishing a diagnosis during the acute stage of illness when therapeutic decisions are critical. PCR to detect distinctive genes from *R. prowazekii* can be used to confirm the diagnosis of epidemic typhus, along with immunofluorescent staining of tissue biopsy specimens. Serology is usually used to identify rickettsial infections. However, adequate antibody titers take up to 10 days to develop. Antibiotic therapy is typically begun before the diagnosis is complete. The most common drugs used to treat patients with epidemic typhus are doxycycline or chloramphenicol.

Murine (Endemic) Typhus

Murine typhus (also known as endemic typhus) is caused by *Rickettsia typhi* and is transmitted by the bite of the rat flea, *Xenopsylla cheopis*, with infected rats as the main reservoir. Clinical signs and symptoms of **murine typhus** include a rash and chills accompanied by headache and fever that last about 12 days. Some patients also exhibit a cough and pneumonia-like symptoms. Severe illness can develop in immunocompromised patients, with seizures, coma, and renal and respiratory failure.

Clinical diagnosis of murine typhus can be confirmed from a biopsy specimen from the rash. Diagnostic tests include indirect immunofluorescent antibody (IFA) staining, PCR for *R. typhi*, and acute and convalescent serologic testing. Primary treatment is doxycycline, with chloramphenicol as the second choice.

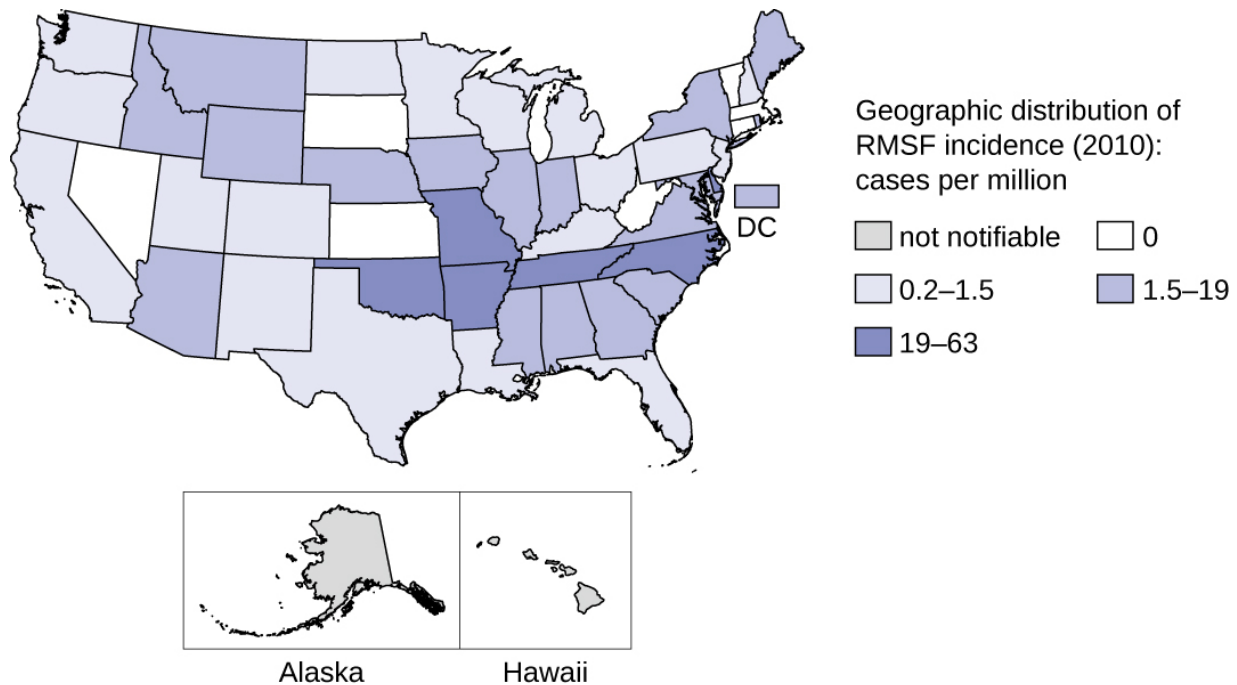
Rocky Mountain Spotted Fever

The disease **Rocky Mountain spotted fever** (RMSF) is caused by *Rickettsia rickettsii* and is transmitted by the bite of a hard-bodied tick such as the American dog tick (*Dermacentor variabilis*), Rocky Mountain wood tick (*D. andersoni*), or brown dog tick (*Rhipicephalus sanguineus*).

This disease is endemic in North and South America and its incidence is coincident with the arthropod vector range. Despite its name, most cases in the US do not occur in the Rocky Mountain region but in the Southeast; North Carolina, Oklahoma, Arkansas, Tennessee, and Missouri account for greater than 60% of all cases.[\[footnote\]](#) The map in [\[link\]](#) shows the distribution of prevalence in the US in 2010.

Centers for Disease Control and Prevention. “Rocky Mountain Spotted Fever (RMSF): Statistics and Epidemiology.”

<http://www.cdc.gov/rmsf/stats/index.html>. Accessed Sept 16, 2016.



In the US, Rocky Mountain spotted fever is most prevalent in the southeastern states. (credit: modification of work by Centers for Disease Control and Prevention)

Signs and symptoms of RMSF include a high fever, headache, body aches, nausea, and vomiting. A petechial rash (similar in appearance to measles) begins on the hands and wrists, and spreads to the trunk, face, and extremities ([link](#)). If untreated, RMSF is a serious illness that can be fatal in the first 8 days even in otherwise healthy patients. Ideally, treatment should begin before petechiae develop, because this is a sign of progression to severe disease; however, the rash usually does not appear until day 6 or later after onset of symptoms and only occurs in 35%–60% of patients with the infection. Increased vascular permeability associated with petechiae formation can result in fatality rates of 3% or greater, even in the presence of clinical support. Most deaths are due to hypotension and cardiac arrest or from ischemia following blood coagulation.

Diagnosis can be challenging because the disease mimics several other diseases that are more prevalent. The diagnosis of RMSF is made based on

symptoms, fluorescent antibody staining of a biopsy specimen from the rash, PCR for *Rickettsia rickettsii*, and acute and convalescent serologic testing. Primary treatment is doxycycline, with chloramphenicol as the second choice.



Rocky Mountain spotted fever causes a petechial rash. Unlike epidemic or murine typhus, the rash begins at the hands and wrists and then spreads to the trunk. (credit: modification of work by Centers for Disease Control and Prevention)

Lyme Disease

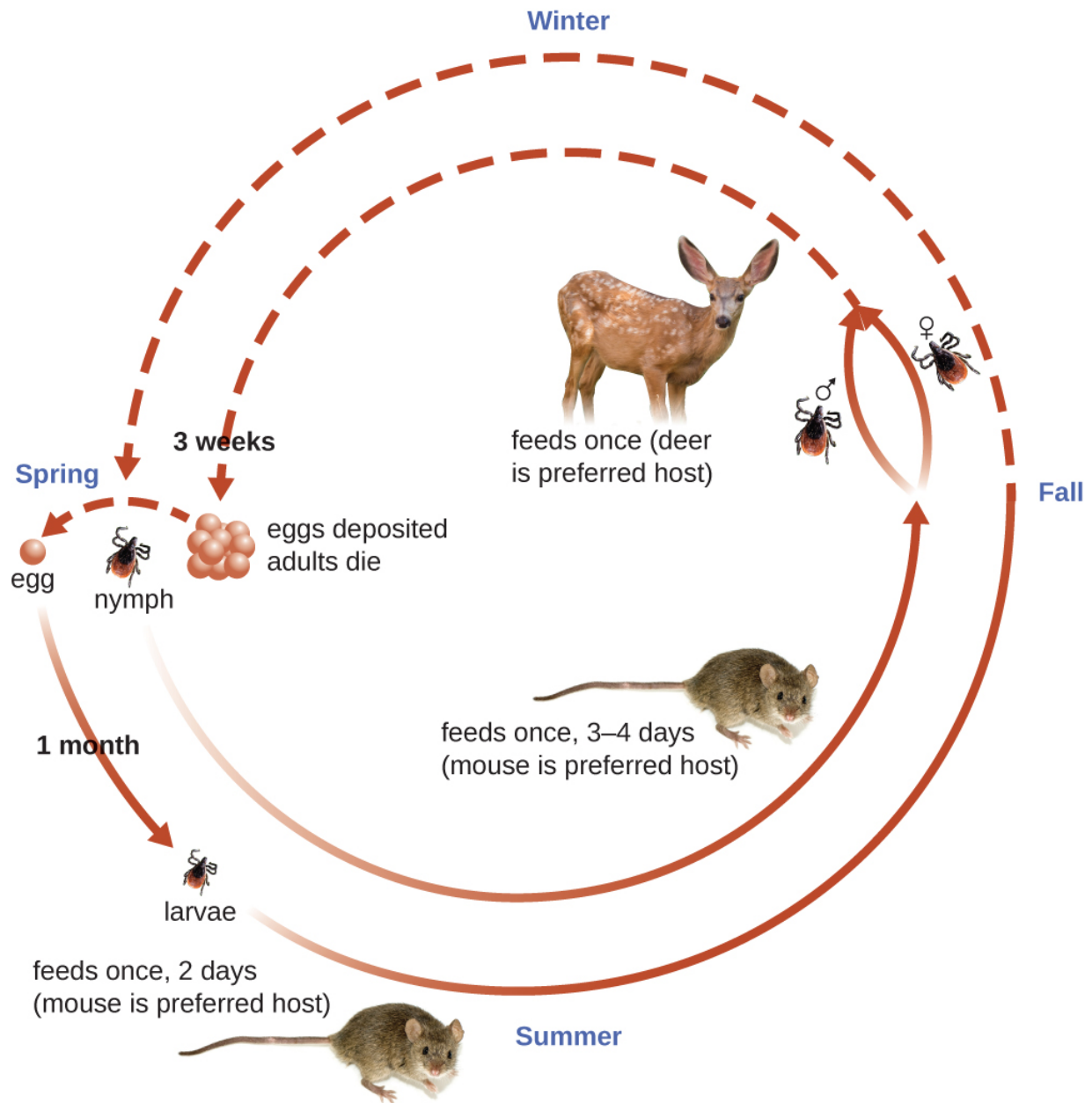
Lyme disease is caused by the spirochete *Borrelia burgdorferi* that is transmitted by the bite of a hard-bodied, black-legged *Ixodes* tick. *I. scapularis* is the biological vector transmitting *B. burgdorferi* in the eastern and north-central US and *I. pacificus* transmits *B. burgdorferi* in the western US ([link](#)). Different species of *Ixodes* ticks are responsible for *B. burgdorferi* transmission in Asia and Europe. In the US, Lyme disease is the

most commonly reported vectorborne illness. In 2014, it was the fifth most common Nationally Notifiable disease.[\[footnote\]](#)

Centers for Disease Control and Prevention. "Lyme Disease. Data and Statistics." 2015. <http://www.cdc.gov/lyme/stats/index.html>. Accessed July 26, 2016.

Ixodes ticks have complex life cycles and deer, mice, and even birds can act as reservoirs. Over 2 years, the ticks pass through four developmental stages and require a blood meal from a host at each stage. In the spring, tick eggs hatch into six-legged larvae. These larvae do not carry *B. burgdorferi* initially. They may acquire the spirochete when they take their first blood meal (typically from a mouse). The larvae then overwinter and molt into eight-legged nymphs in the following spring. Nymphs take blood meals primarily from small rodents, but may also feed on humans, burrowing into the skin. The feeding period can last several days to a week, and it typically takes 24 hours for an infected nymph to transmit enough *B. burgdorferi* to cause infection in a human host. Nymphs ultimately mature into male and female adult ticks, which tend to feed on larger animals like deer or, occasionally, humans. The adults then mate and produce eggs to continue the cycle ([\[link\]](#)).

Life Cycle of the *Ixodes scapularis* Tick



This image shows the 2-year life cycle of the black-legged tick, the biological vector of Lyme disease. (credit "mouse": modification of work by George Shuklin)

The symptoms of Lyme disease follow three stages: early localized, early disseminated, and late stage. During the early-localized stage, approximately 70%–80%^[footnote] of cases may be characterized by a bull's-eye rash, called erythema migrans, at the site of the initial tick bite. The rash forms 3 to 30 days after the tick bite (7 days is the average) and may also be warm to the touch (^[link]).^[footnote] This diagnostic sign is often overlooked if the tick bite occurs on the scalp or another less visible location. Other early symptoms include flu-like symptoms such as malaise, headache, fever, and muscle stiffness. If the patient goes untreated, the second early-disseminated stage of the disease occurs days to weeks later. The symptoms at this stage may include severe headache, neck stiffness, facial paralysis, arthritis, and carditis. The late-stage manifestations of the disease may occur years after exposure. Chronic inflammation causes damage that can eventually cause severe arthritis, meningitis, encephalitis, and altered mental states. The disease may be fatal if untreated.

Centers for Disease Control and Prevention. “Signs and Symptoms of Untreated Lyme Disease.” 2015.

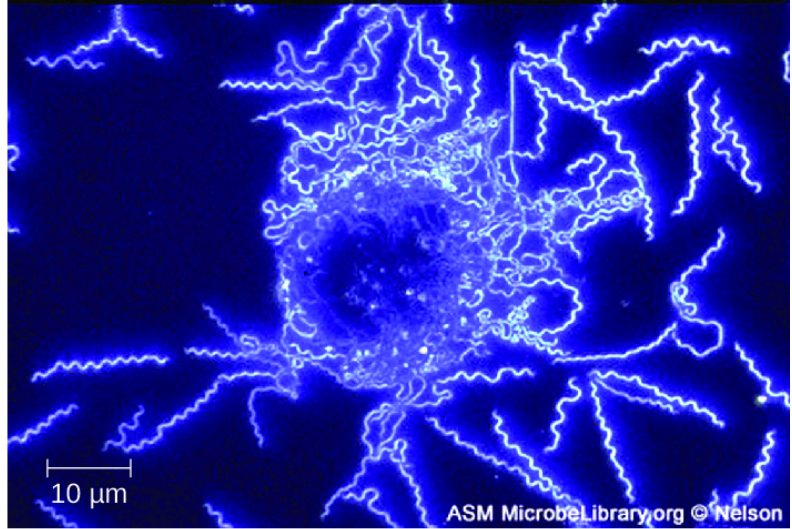
http://www.cdc.gov/lyme/signs_symptoms/index.html. Accessed July 27, 2016.

Centers for Disease Control and Prevention. “Ticks. Symptoms of Tickborne Illness.” 2015. <http://www.cdc.gov/ticks/symptoms.html>. Accessed July 27, 2016.

A presumptive diagnosis of Lyme disease can be made based solely on the presence of a bull's-eye rash at the site of infection, if it is present, in addition to other associated symptoms (^[link]). In addition, indirect immunofluorescent antibody (IFA) labeling can be used to visualize bacteria from blood or skin biopsy specimens. Serological tests like ELISA can also be used to detect serum antibodies produced in response to infection. During the early stage of infection (about 30 days), antibacterial drugs such as amoxicillin and doxycycline are effective. In the later stages, penicillin G, chloramphenicol, or ceftriaxone can be given intravenously.



(a)



(b)

(a) A characteristic bull's eye rash of Lyme disease forms at the site of a tick bite. (b) A darkfield micrograph shows *Borrelia burgdorferi*, the causative agent of Lyme disease. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by American Society for Microbiology)

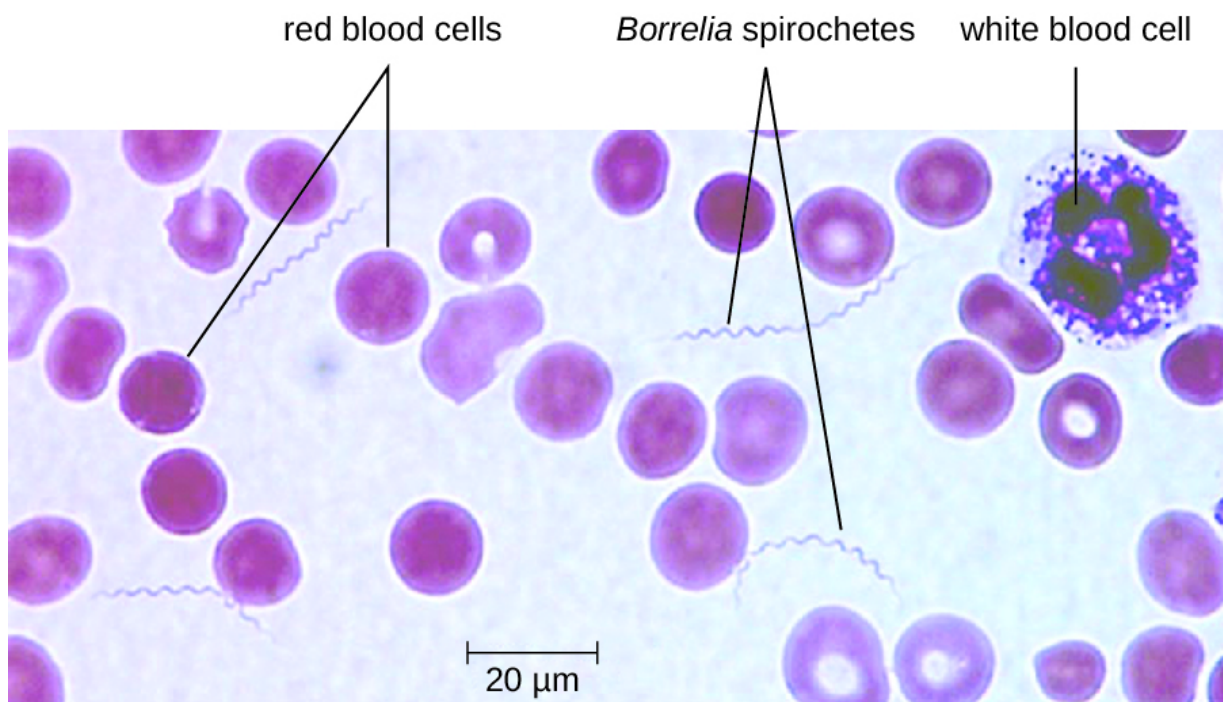
Relapsing Fever

Borrelia spp. also can cause **relapsing fever**. Two of the most common species are *B. recurrentis*, which causes epidemics of louseborne relapsing fever, and *B. hermsii*, which causes tickborne relapsing fevers. These *Borrelia* species are transmitted by the body louse *Pediculus humanus* and the soft-bodied tick *Ornithodoros hermsi*, respectively. Lice acquire the spirochetes from human reservoirs, whereas ticks acquire them from rodent reservoirs. Spirochetes infect humans when *Borrelia* in the vector's saliva or excreta enter the skin rapidly as the vector bites.

In both louse- and tickborne relapsing fevers, bacteremia usually occurs after the initial exposure, leading to a sudden high fever (39–43 °C [102.2–109.4 °F]) typically accompanied by headache and muscle aches. After

about 3 days, these symptoms typically subside, only to return again after about a week. After another 3 days, the symptoms subside again but return a week later, and this cycle may repeat several times unless it is disrupted by antibiotic treatment. Immune evasion through bacterial antigenic variation is responsible for the cyclical nature of the symptoms in these diseases.

The diagnosis of relapsing fever can be made by observation of spirochetes in blood, using darkfield microscopy ([link](#)). For louseborne relapsing fever, doxycycline or erythromycin are the first-line antibiotics. For tickborne relapsing fever, tetracycline or erythromycin are the first-line antibiotics.



A peripheral blood smear from a patient with tickborne relapsing fever. *Borrelia* appears as thin spirochetes among the larger red blood cells. (credit: modification of work by Centers for Disease Control and Prevention)

Trench Fever

The louseborne disease **trench fever** was first characterized as a specific disease during World War I, when approximately 1 million soldiers were infected. Today, it is primarily limited to areas of the developing world where poor sanitation and hygiene lead to infestations of lice (e.g., overpopulated urban areas and refugee camps). Trench fever is caused by the gram-negative bacterium *Bartonella quintana*, which is transmitted when feces from infected body lice, *Pediculus humanus* var *corporis*, are rubbed into the louse bite, abraded skin, or the conjunctiva. The symptoms typically follow a 5-day course marked by a high fever, body aches, conjunctivitis, ocular pain, severe headaches, and severe bone pain in the shins, neck, and back. Diagnosis can be made using blood cultures; serological tests like ELISA can be used to detect antibody titers to the pathogen and PCR can also be used. The first-line antibiotics are doxycycline, macrolide antibiotics, and ceftriaxone.

Note:

- What is the vector associated with epidemic typhus?
- Describe the life cycle of the deer tick and how it spreads Lyme disease.

Note:

Tick Tips

Many of the diseases covered in this chapter involve arthropod vectors. Of these, ticks are probably the most commonly encountered in the US. Adult ticks have eight legs and two body segments, the cephalothorax and the head ([\[link\]](#)). They typically range from 2 mm to 4 mm in length, and feed on the blood of the host by attaching themselves to the skin.

Unattached ticks should be removed and eliminated as soon as they are discovered. When removing a tick that has already attached itself, keep the

following guidelines in mind to reduce the chances of exposure to pathogens:

- Use blunt tweezers to gently pull near the site of attachment until the tick releases its hold on the skin.
- Avoid crushing the tick's body and do not handle the tick with bare fingers. This could release bacterial pathogens and actually increase your exposure. The tick can be killed by drowning in water or alcohol, or frozen if it may be needed later for identification and analysis.
- Disinfect the area thoroughly by swabbing with an antiseptic such as isopropanol.
- Monitor the site of the bite for rashes or other signs of infection.

Many ill-advised home remedies for tick removal have become popular in recent years, propagated by social media and pseudojournalism. Health professionals should discourage patients from resorting to any of the following methods, which are NOT recommended:

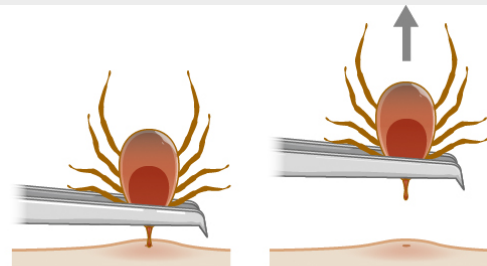
- using chemicals (e.g., petroleum jelly or fingernail polish) to dislodge an attached tick, because it can cause the tick to release fluid, which can increase the chance of infection
- using hot objects (matches or cigarette butts) to dislodge an attached tick
- squeezing the tick's body with fingers or tweezers



(a)



(b)



(c)

(a) This black-legged tick, also known as the deer tick, has not yet attached to the skin. (b) A notched tick extractor can be used for removal. (c) To remove an attached tick with fine-tipped tweezers, pull gently on the mouth parts until the tick releases its hold on the skin. Avoid squeezing the tick's body, because this could release

pathogens and thus increase the risk of contracting Lyme disease.
(credit a: modification of work by Jerry Kirkhart; credit c:
modification of work by Centers for Disease Control and Prevention)

Note:

Bacterial Infections of the Circulatory and Lymphatic Systems

Although the circulatory system is a closed system, bacteria can enter the bloodstream through several routes. Wounds, animal bites, or other breaks in the skin and mucous membranes can result in the rapid dissemination of bacterial pathogens throughout the body. Localized infections may also spread to the bloodstream, causing serious and often fatal systemic infections. [\[link\]](#) and [\[link\]](#) summarize the major characteristics of bacterial infections of the circulatory and lymphatic systems.

Bacterial Infections of the Circulatory and Lymphatic Systems					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Anaplasmosis (HGA)	<i>Anaplasma phagocytophilum</i>	Fever, flu-like symptoms	From small-mammal reservoirs via tick vector	Blood smear, PCR	Doxycycline
Brucellosis	<i>Brucella melitensis</i> , <i>B. abortus</i> , <i>B. canis</i> , <i>B. suis</i>	Granuloma, undulating fever, chronic flu-like symptoms	Direct contact with infected livestock or animals	Agglutination tests, ELISA	Doxycycline, rifampin
Cat-scratch disease	<i>Bartonella henselae</i>	Lymph-node swelling and pain, fever, chills, fatigue	Bite or scratch from domestic cats	Immunofluorescence, serological tests, PCR	None for immunocompetent patients
Ehrlichiosis (HME)	<i>Ehrlichia chaffeensis</i>	Flu-like symptoms, rash	Lone star tick vector	Serologic tests, PCR	Doxycycline
Endocarditis/pericarditis	<i>Staphylococcus</i> spp., <i>Streptococcus</i> spp., <i>Enterococcus</i> spp., HACEK bacilli	Chest pain, difficulty breathing, dry cough, fever; potentially fatal damage to heart valves	Pathogens introduced to bloodstream via contaminated catheters, dental procedures, piercings, or wounds	Echocardiogram, blood culture	Ampicillin, nafcillin, gentamicin, others; based on susceptibility testing
Epidemic typhus	<i>Rickettsia prowazekii</i>	High fever, body aches, rash; potentially fatal damage to heart and brain	From rodent reservoir via body louse vector	PCR, immunofluorescence	Doxycycline, chloramphenicol
Gas gangrene	<i>Clostridium perfringens</i> , other <i>Clostridium</i> spp.	Rapidly spreading myonecrosis, edema, yellowish and then purple discharge from wound, pockets of gas in tissues, septic shock and death	Germination of endospores in ischemic tissues, typically due to injury or chronic disease (e.g., diabetes)	Wound culture	Penicillin G, clindamycin, metronidazole
Infectious arthritis (septic arthritis)	<i>Staphylococcus aureus</i> , <i>Neisseria gonorrhoeae</i>	Joint pain and swelling, limited range of motion	Infection spreads to joint via circulatory system from wound or surgical site	Synovial fluid culture	Oxacillin, cefazolin, cephtriaxone
Lyme disease	<i>Borrelia burgdorferi</i>	Early localized: bull's eye rash, malaise, headache, fever, muscle stiffness; early disseminated: stiff neck, facial paralysis, arthritis, carditis; late-stage: arthritis, meningitis, possibly fatal	From deer, rodent, bird reservoirs via tick vector	IFA, serology, and ELISA	Amoxicillin, doxycycline, penicillin G, chloramphenicol, ceftriaxone
Murine (endemic) typhus	<i>Rickettsia typhi</i>	Low-grade fever, rash, headache, cough	From rodents or between humans via rat flea vector	Biopsy, IFA, PCR	Doxycycline, chloramphenicol
Osteomyelitis	<i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> , others	Inflammation of bone tissue, leading to fever, localized pain, edema, ulcers, bone loss	Pathogens introduced through trauma, prosthetic joint replacement, or from other infected body site via bloodstream	Radiograph of affected bone, culture of bone biopsy specimen	Cephalosporin, penicillins, others

Bacterial Infections of the Circulatory and Lymphatic Systems (continued)					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Plague	<i>Yersinia pestis</i>	Bubonic: buboes, fever, internal hemorrhaging; septicemic: fever, abdominal pain, shock, DIC, necrosis in extremities; pneumonic: acute pneumonia, respiratory failure, shock. All forms have high mortality rates.	Transmitted from mammal reservoirs via flea vectors or consumption of infected animal; transmission of pneumonic plague between humans via respiratory aerosols	Culture of bacteria from lymph, blood, or sputum samples; DFA, ELISA	Gentamycin, fluoroquinolones, others
Puerperal sepsis	<i>Streptococcus pyogenes</i> , many others	Rapid-onset fever, shock, and death	Pathogens introduced during or immediately following childbirth	Wound, urine, or blood culture	As determined by susceptibility testing
Rat-bite fever	<i>Streptobacillus moniliformis</i> , <i>Spirillum minor</i>	Fever, muscle and joint pain, rash, ulcer	Bite from infected rat or exposure to rat feces or body fluids in contaminated food or water	Observation of the organism from samples and antibody tests	Penicillin
Relapsing fever	<i>Borrelia recurrentis</i> , <i>B. hermsii</i> , other <i>Borrelia</i> spp.	Recurring fever, headache, muscle aches	From rodent or human reservoir via body louse or tick vector	Darkfield microscopy	Doxycycline, tetracycline, erythromycin
Rheumatic fever	<i>Streptococcus pyogenes</i>	Joint pain and swelling, inflammation and scarring of heart valves, heart murmur	Sequela of streptococcal pharyngitis	Serology, electrocardiogram, echocardiogram	Benzathine benzylpenicillin
Rocky Mountain spotted fever	<i>Rickettsia rickettsii</i>	High fever, headache, body aches, nausea and vomiting, petechial rash; potentially fatal hypotension and ischemia due to blood coagulation	From rodent reservoir via tick vectors	Biopsy, serology, PCR	Doxycycline, chloramphenicol
Toxic shock syndrome (TSS)	<i>Staphylococcus aureus</i>	Sudden high fever, vomiting, diarrhea, hypotension, death	Pathogens from localized infection spread to bloodstream; pathogens introduced on tampons or other intravaginal products	Serology, toxin identification from isolates	Clindamycin, vancomycin
Toxic shock-like syndrome (STSS)	<i>Streptococcus pyogenes</i>	Sudden high fever, vomiting, diarrhea, acute respiratory distress syndrome (ARDS), hypoxemia, necrotizing fasciitis, death	Sequela of streptococcal skin or soft-tissue infection	Serology, blood culture, urinalysis	Penicillin, cephalosporin
Trench fever	<i>Bartonella quintana</i>	High fever, conjunctivitis, ocular pain, headaches, severe pain in bones of shins, neck, and back	Between humans via body louse vector	Blood culture, ELISA, PCR	Doxycycline, macrolide antibiotics, ceftriaxone
Tularemia (rabbit fever)	<i>Francisella tularensis</i>	Skin lesions, fever, chills, headache, buboes	Eating or handling infected rabbit; transmission from infected animal via tick or fly vector; aerosol transmission (in laboratory or as bioweapon)	DFA	Streptomycin, gentamycin, others

Key Concepts and Summary

- Bacterial infections of the circulatory system are almost universally serious. Left untreated, most have high mortality rates.
- Bacterial pathogens usually require a breach in the immune defenses to colonize the circulatory system. Most often, this involves a wound or the bite of an arthropod vector, but it can also occur in hospital settings and result in nosocomial infections.
- **Sepsis** from both gram-negative and gram-positive bacteria, **puerperal fever**, **rheumatic fever**, **endocarditis**, **gas gangrene**, **osteomyelitis**, and **toxic shock syndrome** are typically a result of injury or introduction of bacteria by medical or surgical intervention.
- **Tularemia**, **brucellosis**, **cat-scratch fever**, **rat-bite fever**, and **bubonic plague** are zoonotic diseases transmitted by biological vectors
- **Ehrlichiosis**, **anaplasmosis**, **endemic** and **murine typhus**, **Rocky Mountain spotted fever**, **Lyme disease**, **relapsing fever**, and **trench fever** are transmitted by arthropod vectors.
- Because their symptoms are so similar to those of other diseases, many bacterial infections of the circulatory system are difficult to diagnose.
- Standard antibiotic therapies are effective for the treatment of most bacterial infections of the circulatory system, unless the bacterium is resistant, in which case synergistic treatment may be required.
- The systemic immune response to a bacteremia, which involves the release of excessive amounts of cytokines, can sometimes be more damaging to the host than the infection itself.

Multiple Choice

Exercise:

Problem: Which of the following diseases is caused by a spirochete?

- A. tularemia
- B. relapsing fever
- C. rheumatic fever
- D. Rocky Mountain spotted fever

Solution:

B

Exercise:

Problem: Which of the following diseases is transmitted by body lice?

- A. tularemia
- B. bubonic plague
- C. murine typhus
- D. epidemic typhus

Solution:

D

Exercise:

Problem:

What disease is most associated with *Clostridium perfringens*?

- A. endocarditis
- B. osteomyelitis
- C. gas gangrene
- D. rat bite fever

Solution:

C

Exercise:

Problem: Which bacterial pathogen causes plague?

- A. *Yersinia pestis*
- B. *Bacillus moniliformis*
- C. *Bartonella quintana*
- D. *Rickettsia rickettsii*

Solution:

A

Fill in the Blank

Exercise:

Problem:

Lyme disease is characterized by a(n) _____ that forms at the site of infection.

Solution:

bull's eye-rash

Exercise:

Problem:

_____ refers to a loss of blood pressure resulting from a system-wide infection.

Solution:

Septic shock

Short Answer

Exercise:

Problem:

What are the three forms of plague and how are they contracted?

Exercise:

Problem: Compare epidemic and murine typhus.

Critical Thinking

Exercise:

Problem:

Why are most vascular pathogens poorly communicable from person to person?

Exercise:

Problem:

How have human behaviors contributed to the spread or control of arthropod-borne vascular diseases?

Viral Infections of the Circulatory and Lymphatic Systems

LEARNING OBJECTIVES

- Identify common viral pathogens that cause infections of the circulatory and lymphatic systems
- Compare the major characteristics of specific viral diseases affecting the circulatory and lymphatic systems

Viral pathogens of the circulatory system vary tremendously both in their virulence and distribution worldwide. Some of these pathogens are practically global in their distribution. Fortunately, the most ubiquitous viruses tend to produce the mildest forms of disease. In the majority of cases, those infected remain asymptomatic. On the other hand, other viruses are associated with life-threatening diseases that have impacted human history.

Infectious Mononucleosis and Burkitt Lymphoma

Human herpesvirus 4, also known as Epstein-Barr virus (EBV), has been associated with a variety of human diseases, such as mononucleosis and Burkitt lymphoma. Exposure to the human herpesvirus 4 (HHV-4) is widespread and nearly all people have been exposed at some time in their childhood, as evidenced by serological tests on populations. The virus primarily resides within B lymphocytes and, like all herpes viruses, can remain dormant in a latent state for a long time.

When uninfected young adults are exposed to EBV, they may experience **infectious mononucleosis**. The virus is mainly spread through contact with

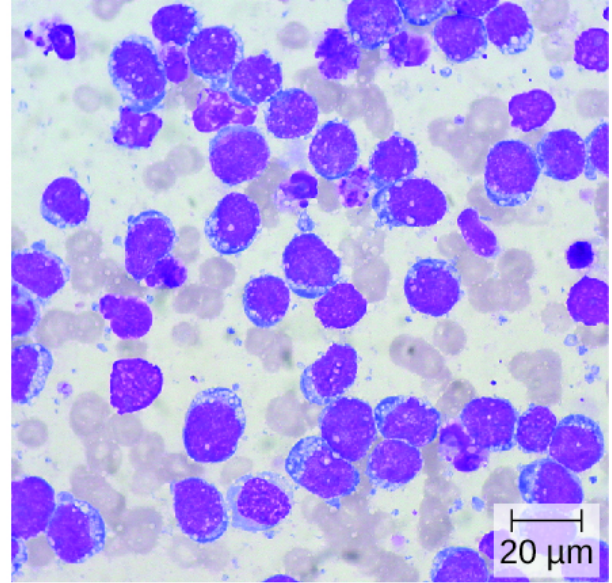
body fluids (e.g., saliva, blood, and semen). The main symptoms include pharyngitis, fever, fatigue, and lymph node swelling. Abdominal pain may also occur as a result of spleen and liver enlargement in the second or third week of infection. The disease typically is self-limiting after about a month. The main symptom, extreme fatigue, can continue for several months, however. Complications in immunocompetent patients are rare but can include jaundice, anemia, and possible rupture of the spleen caused by enlargement.

In patients with malaria or HIV, Epstein-Barr virus can lead to a fast-growing malignant cancer known as **Burkitt lymphoma** ([\[link\]](#)). This condition is a form of non-Hodgkin lymphoma that produces solid tumors chiefly consisting of aberrant B cells. Burkitt lymphoma is more common in Africa, where prevalence of HIV and malaria is high, and it more frequently afflicts children. Repeated episodes of viremia caused by reactivation of the virus are common in immunocompromised individuals. In some patients with AIDS, EBV may induce the formation of malignant B-cell lymphomas or oral hairy leukoplakia. Immunodeficiency-associated Burkitt lymphoma primarily occurs in patients with HIV. HIV infection, similar to malaria, leads to polyclonal B-cell activation and permits poorly controlled proliferation of EBV⁺ B cells, leading to the formation of lymphomas.

Infectious mononucleosis is typically diagnosed based on the initial clinical symptoms and a test for antibodies to EBV-associated antigens. Because the disease is self-limiting, antiviral treatments are rare for mononucleosis. Cases of Burkitt lymphoma are diagnosed from a biopsy specimen from a lymph node or tissue from a suspected tumor. Staging of the cancer includes computed tomography (CT) scans of the chest, abdomen, pelvis, and cytologic and histologic evaluation of biopsy specimens. Because the tumors grow so rapidly, staging studies must be expedited and treatment must be initiated promptly. An intensive alternating regimen of cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, and cytarabine (CODOX-M/IVAC) plus rituximab results in a cure rate greater than 90% for children and adults.



(a)



(b)

(a) Burkitt lymphoma can cause large tumors. (b) Characteristic irregularly shaped abnormal lymphocytes (large purple cells) with vacuoles (white spots) from a fine-needle aspirate of a tumor from a patient with Burkitt lymphoma. (credit a: modification of work by Bi CF, Tang Y, Zhang WY, Zhao S, Wang XQ, Yang QP, Li GD, and Liu WP; credit b: modification of work by Ed Uthman)

Cytomegalovirus Infections

Also known as cytomegalovirus (CMV), human herpesvirus 5 (HHV-5) is a virus with high infection rates in the human population. It is currently estimated that 50% of people in the US have been infected by the time they reach adulthood.[\[footnote\]](#) CMV is the major cause of non-Epstein-Barr infectious mononucleosis in the general human population. It is also an important pathogen in immunocompromised hosts, including patients with AIDS, neonates, and transplant recipients. However, the vast majority of CMV infections are asymptomatic. In adults, if symptoms do occur, they typically include fever, fatigue, swollen glands, and pharyngitis.

Centers for Disease Control and Prevention. “Cytomegalovirus (CMV) and Congenital CMV Infection: About CMV.” 2016.

<http://www.cdc.gov/cmV/transmission.html>. Accessed July 28, 2016.

CMV can be transmitted between individuals through contact with body fluids such as saliva or urine. Common modes of transmission include sexual contact, nursing, blood transfusions, and organ transplants. In addition, pregnant women with active infections frequently pass this virus to their fetus, resulting in congenital CMV infections, which occur in approximately one in every 150 infants in US.^[footnote] Infants can also be infected during passage through the birth canal or through breast milk and saliva from the mother.

Centers for Disease Control and Prevention. “Cytomegalovirus (CMV) and Congenital CMV Infection: Babies Born with CMV (Congenital CMV Infection).” 2016. <http://www.cdc.gov/cmV/congenital-infection.html>. Accessed July 28, 2016.

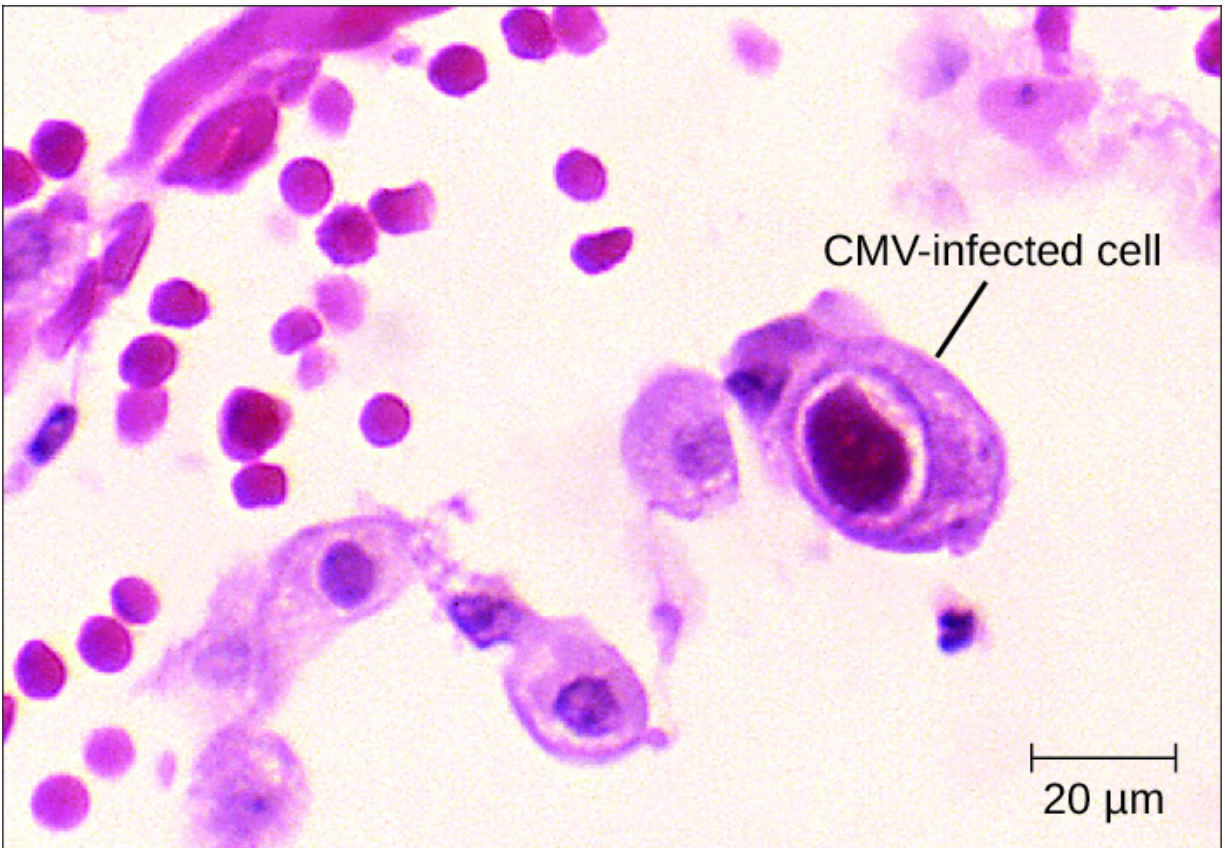
Perinatal infections tend to be milder but can occasionally cause lung, spleen, or liver damage. Serious symptoms in newborns include growth retardation, jaundice, deafness, blindness, and mental retardation if the virus crosses the placenta during the embryonic state when the body systems are developing in utero. However, a majority (approximately 80%) of infected infants will never have symptoms or experience long-term problems.^[footnote] Diagnosis of CMV infection during pregnancy is usually achieved by serology; CMV is the “C” in prenatal TORCH screening. *ibid.*

Many patients receiving blood transfusions and nearly all those receiving kidney transplants ultimately become infected with CMV. Approximately 60% of transplant recipients will have CMV infection and more than 20% will develop symptomatic disease.^[footnote] These infections may result from CMV-contaminated tissues but also may be a consequence of immunosuppression required for transplantation causing reactivation of prior CMV infections. The resulting viremia can lead to fever and leukopenia, a decrease in the number of white blood cells in the bloodstream. Serious consequences may include liver damage, transplant rejection, and death. For similar reasons, many patients with AIDS develop active CMV infections that can manifest as encephalitis or progressive retinitis leading to blindness.^[footnote]

E. Cordero et al. "Cytomegalovirus Disease in Kidney Transplant Recipients: Incidence, Clinical Profile, and Risk Factors." *Transplantation Proceedings* 44 no. 3 (2012):694–700.

L.M. Mofenson et al. "Guidelines for the Prevention and Treatment of Opportunistic Infections Among HIV-Exposed and HIV-Infected Children: Recommendations From CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics." *MMWR Recommendations and Reports* 58 no. RR-11 (2009):1–166.

Diagnosis of a localized CMV infection can be achieved through direct microscopic evaluation of tissue specimens stained with routine stains (e.g., Wright-Giemsa, hematoxylin and eosin, Papanicolaou) and immunohistochemical stains. Cells infected by CMV produce characteristic inclusions with an "owl's eye" appearance; this sign is less sensitive than molecular methods like PCR but more predictive of localized disease ([link](#)). For more severe CMV infection, tests such as enzyme immunoassay (EIA), indirect immunofluorescence antibody (IFA) tests, and PCR, which are based on detection of CMV antigen or DNA, have a higher sensitivity and can determine viral load. Cultivation of the virus from saliva or urine is still the method for detecting CMV in newborn babies up to 3 weeks old. Ganciclovir, valganciclovir, foscarnet, and cidofovir are the first-line antiviral drugs for serious CMV infections.



Cells infected with CMV become enlarged and have a characteristic “owl’s eye” nucleus. This micrograph shows kidney cells from a patient with CMV. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- Compare the diseases caused by HHV-4 and HHV-5.

Arthropod-Borne Viral Diseases

There are a number of arthropod-borne viruses, or **arboviruses**, that can cause human disease. Among these are several important hemorrhagic fevers transmitted by mosquitoes. We will discuss three that pose serious threats: yellow fever, chikungunya fever, and dengue fever.

Yellow Fever

Yellow fever was once common in the US and caused several serious outbreaks between 1700 and 1900.[\[footnote\]](#) Through vector control efforts, however, this disease has been eliminated in the US. Currently, **yellow fever** occurs primarily in tropical and subtropical areas in South America and Africa. It is caused by the yellow fever virus of the genus *Flavivirus* (named for the Latin word *flavus* meaning *yellow*), which is transmitted to humans by mosquito vectors. Sylvatic yellow fever occurs in tropical jungle regions of Africa and Central and South America, where the virus can be transmitted from infected monkeys to humans by the mosquitoes *Aedes africanus* or *Haemagogus* spp. In urban areas, the *Aedes aegypti* mosquito is mostly responsible for transmitting the virus between humans.

Centers for Disease Control and Prevention. “History Timeline Transcript.” <http://www.cdc.gov/travel-training/local/HistoryEpidemiologyandVaccination/HistoryTimelineTranscript.pdf>. Accessed July 28, 2016.

Most individuals infected with yellow fever virus have no illness or only mild disease. Onset of milder symptoms is sudden, with dizziness, fever of 39–40 °C (102–104 °F), chills, headache, and myalgias. As symptoms worsen, the face becomes flushed, and nausea, vomiting, constipation, severe fatigue, restlessness, and irritability are common. Mild disease may resolve after 1 to 3 days. However, approximately 15% of cases progress to develop moderate to severe yellow fever disease.[\[footnote\]](#)

Centers for Disease Control and Prevention. “Yellow Fever, Symptoms and Treatment.” 2015 <http://www.cdc.gov/yellowfever/symptoms/index.html>. Accessed July 28, 2016.

In moderate or severe disease, the fever falls suddenly 2 to 5 days after onset, but recurs several hours or days later. Symptoms of jaundice,

petechial rash, mucosal hemorrhages, oliguria (scant urine), epigastric tenderness with bloody vomit, confusion, and apathy also often occur for approximately 7 days of moderate to severe disease. After more than a week, patients may have a rapid recovery and no sequelae.

In its most severe form, called malignant yellow fever, symptoms include delirium, bleeding, seizures, shock, coma, and multiple organ failure; in some cases, death occurs. Patients with malignant yellow fever also become severely immunocompromised, and even those in recovery may become susceptible to bacterial superinfections and pneumonia. Of the 15% of patients who develop moderate or severe disease, up to half may die.

Diagnosis of yellow fever is often based on clinical signs and symptoms and, if applicable, the patient's travel history, but infection can be confirmed by culture, serologic tests, and PCR. There are no effective treatments for patients with yellow fever. Whenever possible, patients with yellow fever should be hospitalized for close observation and given supportive care. Prevention is the best method of controlling yellow fever. Use of mosquito netting, window screens, insect repellents, and insecticides are all effective methods of reducing exposure to mosquito vectors. An effective vaccine is also available, but in the US, it is only administered to those traveling to areas with endemic yellow fever. In West Africa, the World Health Organization (WHO) launched a Yellow Fever Initiative in 2006 and, since that time, significant progress has been made in combating yellow fever. More than 105 million people have been vaccinated, and no outbreaks of yellow fever were reported in West Africa in 2015.

Note:**Yellow Fever: Altering the Course of History**

Yellow fever originated in Africa and is still most prevalent there today. This disease is thought to have been translocated to the Americas by the slave trade in the 16th century.[\[footnote\]](#) Since that time, yellow fever has been associated with many severe outbreaks, some of which had important impacts upon historic events.

J.T. Cathey, J.S. Marr. "Yellow fever, Asia and the East African Slave Trade." *Transactions of the Royal Society of Tropical Medicine and*

Hygiene 108, no. 5 (2014):252–257.

Yellow fever virus was once an important cause of disease in the US. In the summer of 1793, there was a serious outbreak in Philadelphia (then the US capitol). It is estimated that 5,000 people (10% of the city's population) died. All of the government officials, including George Washington, fled the city in the face of this epidemic. The disease only abated when autumn frosts killed the mosquito vector population.

In 1802, Napoleon Bonaparte sent an army of 40,000 to Hispaniola to suppress a slave revolution. This was seen by many as a part of a plan to use the Louisiana Territory as a granary as he reestablished France as a global power. Yellow fever, however, decimated his army and they were forced to withdraw. Abandoning his aspirations in the New World, Napoleon sold the Louisiana Territory to the US for \$15 million in 1803. The most famous historic event associated with yellow fever is probably the construction of the Panama Canal. The French began work on the canal in the early 1880s. However, engineering problems, malaria, and yellow fever forced them to abandon the project. The US took over the task in 1904 and opened the canal a decade later. During those 10 years, yellow fever was a constant adversary. In the first few years of work, greater than 80% of the American workers in Panama were hospitalized with yellow fever. It was the work of Carlos Finlay and Walter Reed that turned the tide. Taken together, their work demonstrated that the disease was transmitted by mosquitoes. Vector control measures succeeded in reducing both yellow fever and malaria rates and contributed to the ultimate success of the project.

Dengue Fever

The disease **dengue fever**, also known as breakbone fever, is caused by four serotypes of dengue virus called dengue 1–4. These are *Flavivirus* species that are transmitted to humans by *A. aegypti* or *A. albopictus* mosquitoes. The disease is distributed worldwide but is predominantly located in tropical regions. The WHO estimates that 50 million to 100 million infections occur yearly, including 500,000 dengue hemorrhagic fever (DHF) cases and 22,000 deaths, most among children. [\[footnote\]](#)

Dengue fever is primarily a self-limiting disease characterized by abrupt onset of high fever up to 40 °C (104 °F), intense headaches, rash, slight nose or gum bleeding, and extreme muscle, joint, and bone pain, causing patients to feel as if their bones are breaking, which is the reason this disease is also referred to as breakbone fever. As the body temperature returns to normal, in some patients, signs of dengue hemorrhagic fever may develop that include drowsiness, irritability, severe abdominal pain, severe nose or gum bleeding, persistent vomiting, vomiting blood, and black tarry stools, as the disease progresses to DHF or dengue shock syndrome (DSS). Patients who develop DHF experience circulatory system failure caused by increased blood vessel permeability. Patients with dengue fever can also develop DSS from vascular collapse because of the severe drop in blood pressure. Patients who develop DHF or DSS are at greater risk for death without prompt appropriate supportive treatment. About 30% of patients with severe hemorrhagic disease with poor supportive treatment die, but mortality can be less than 1% with experienced support.[\[footnote\]](#)

Centers for Disease Control and Prevention. “Dengue, Epidemiology.” 2014. <http://www.cdc.gov/dengue/epidemiology/index.html>. Accessed July 28, 2016.

C.R. Pringle “Dengue.” MSD Manual: Consumer Version. <https://www.msdmanuals.com/home/infections/viral-infections/dengue>. 2016. Accessed Sept 15, 2016.

Diagnostic tests for dengue fever include serologic testing, ELISA, and reverse transcriptase-polymerase chain reaction (RT-PCR) of blood. There are no specific treatments for dengue fever, nor is there a vaccine. Instead, supportive clinical care is provided to treat the symptoms of the disease. The best way to limit the impact of this viral pathogen is vector control.

Chikungunya Fever

The arboviral disease **chikungunya fever** is caused by chikungunya virus (CHIKV), which is transmitted to humans by *A. aegypti* and *A. albopictus* mosquitoes. Until 2013, the disease had not been reported outside of Africa, Asia, and a few European countries; however, CHIKV has now spread to mosquito populations in North and South America. Chikungunya fever is

characterized by high fever, joint pain, rash, and blisters, with joint pain persisting for several months. These infections are typically self-limiting and rarely fatal.

The diagnostic approach for chikungunya fever is similar to that for dengue fever. Viruses can be cultured directly from patient serum during early infections. IFA, EIA, ELISA, PCR, and RT-PCR are available to detect CHIKV antigens and patient antibody response to the infection. There are no specific treatments for this disease except to manage symptoms with fluids, analgesics, and bed rest. As with most arboviruses, the best strategy for combating the disease is vector control.

Note:

Use this [interactive map](#) to explore the global distribution of dengue.

Note:

- Name three arboviral diseases and explain why they are so named.
- What is the best method for controlling outbreaks of arboviral diseases?

Ebola Virus Disease

The Ebola virus disease (EVD) is a highly contagious disease caused by species of *Ebolavirus*, a BSL-4 filovirus ([\[link\]](#)). Transmission to humans occurs through direct contact with body fluids (e.g., blood, saliva, sweat, urine, feces, or vomit), and indirect contact by contaminated fomites. Infected patients can easily transmit Ebola virus to others if appropriate containment and use of personal protective equipment is not available or used. Handling and working with patients with EVD is extremely hazardous to the general population and health-care workers. In almost every EVD

outbreak there have been Ebola infections among health-care workers. This ease of Ebola virus transmission was recently demonstrated in the Ebola epidemic in Guinea, Liberia, and Sierra Leone in 2014, in which more than 28,000 people in 10 countries were infected and more than 11,000 died.

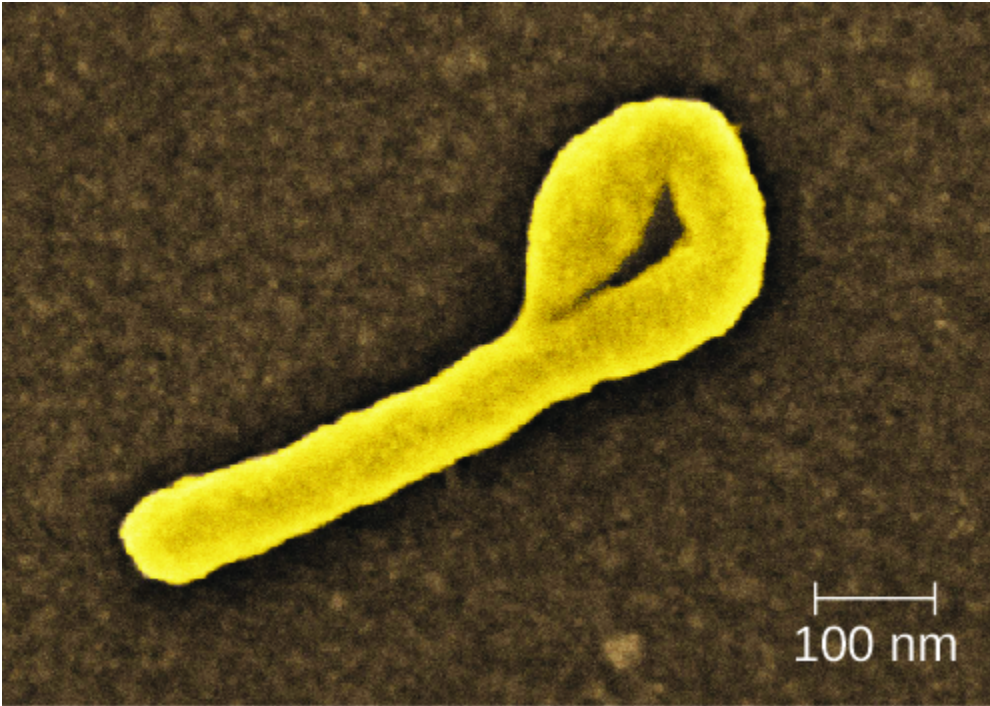
[\[footnote\]](#)

HealthMap. “2014 Ebola Outbreaks.”

<http://www.healthmap.org/ebola/#timeline>. Accessed July 28, 2016.

After infection, the initial symptoms of Ebola are unremarkable: fever, severe headache, myalgia, cough, chest pain, and pharyngitis. As the disease progresses, patients experience abdominal pain, diarrhea, and vomiting. Hemorrhaging begins after about 3 days, with bleeding occurring in the gastrointestinal tract, skin, and many other sites. This often leads to delirium, stupor, and coma, accompanied by shock, multiple organ failure, and death. The mortality rates of EVD often range from 50% to 90%.

The initial diagnosis of Ebola is difficult because the early symptoms are so similar to those of many other illnesses. It is possible to directly detect the virus from patient samples within a few days after symptoms begin, using antigen-capture ELISA, immunoglobulin M (IgM) ELISA, PCR, and virus isolation. There are currently no effective, approved treatments for Ebola other than supportive care and proper isolation techniques to contain its spread.



An Ebola virus particle viewed with electron microscopy. These filamentous viruses often exhibit looped or hooked ends. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- How is Ebola transmitted?

Hantavirus

The genus *Hantavirus* consists of at least four serogroups with nine viruses causing two major clinical (sometimes overlapping) syndromes: **hantavirus pulmonary syndrome** (HPS) in North America and **hemorrhagic fever**

with renal syndrome (HFRS) in other continents. Hantaviruses are found throughout the world in wild rodents that shed the virus in their urine and feces. Transmission occurs between rodents and to humans through inhalation of aerosols of the rodent urine and feces. Hantaviruses associated with outbreaks in the US and Canada are transmitted by the deer mouse, white-footed mouse, or cotton rat.

HPS begins as a nonspecific flu-like illness with headache, fever, myalgia, nausea, vomiting, diarrhea, and abdominal pain. Patients rapidly develop pulmonary edema and hypotension resulting in pneumonia, shock, and death, with a mortality rate of up to 50%.[\[footnote\]](#) This virus can also cause HFRS, which has not been reported in the US. The initial symptoms of this condition include high fever, headache, chills, nausea, inflammation or redness of the eyes, or a rash. Later symptoms are hemorrhaging, hypotension, kidney failure, shock, and death. The mortality rate of HFRS can be as high as 15%.[\[footnote\]](#)

World Health Organization. “Hantavirus Diseases.” 2016.

<http://www.who.int/ith/diseases/hantavirus/en/>. Accessed July 28, 2016.
ibid.

ELISA, Western blot, rapid immunoblot strip assay (RIBA), and RT-PCR detect host antibodies or viral proteins produced during infection.

Immunohistological staining may also be used to detect the presence of viral antigens. There are no clinical treatments other than general supportive care available for HPS infections. Patients with HFRS can be treated with ribavirin.[\[footnote\]](#)

Centers for Disease Control and Prevention. “Hantavirus: Treatment.” 2012. <http://www.cdc.gov/hantavirus/technical/hps/treatment.html>. Accessed July 28, 2016.

Note:

- Compare the two Hantavirus diseases discussed in this section.

Human Immunodeficiency Virus

Human T-lymphotropic viruses (HTLV), also called human immunodeficiency viruses (HIV) are retroviruses that are the causative agent of acquired immune deficiency syndrome (AIDS). There are two main variants of **human immunodeficiency virus (HIV)**. HIV-1 ([\[link\]](#)) occurs in human populations worldwide, whereas HIV-2 is concentrated in West Africa. Currently, the most affected region in the world is sub-Saharan Africa, with an estimated 25.6 million people living with HIV in 2015.

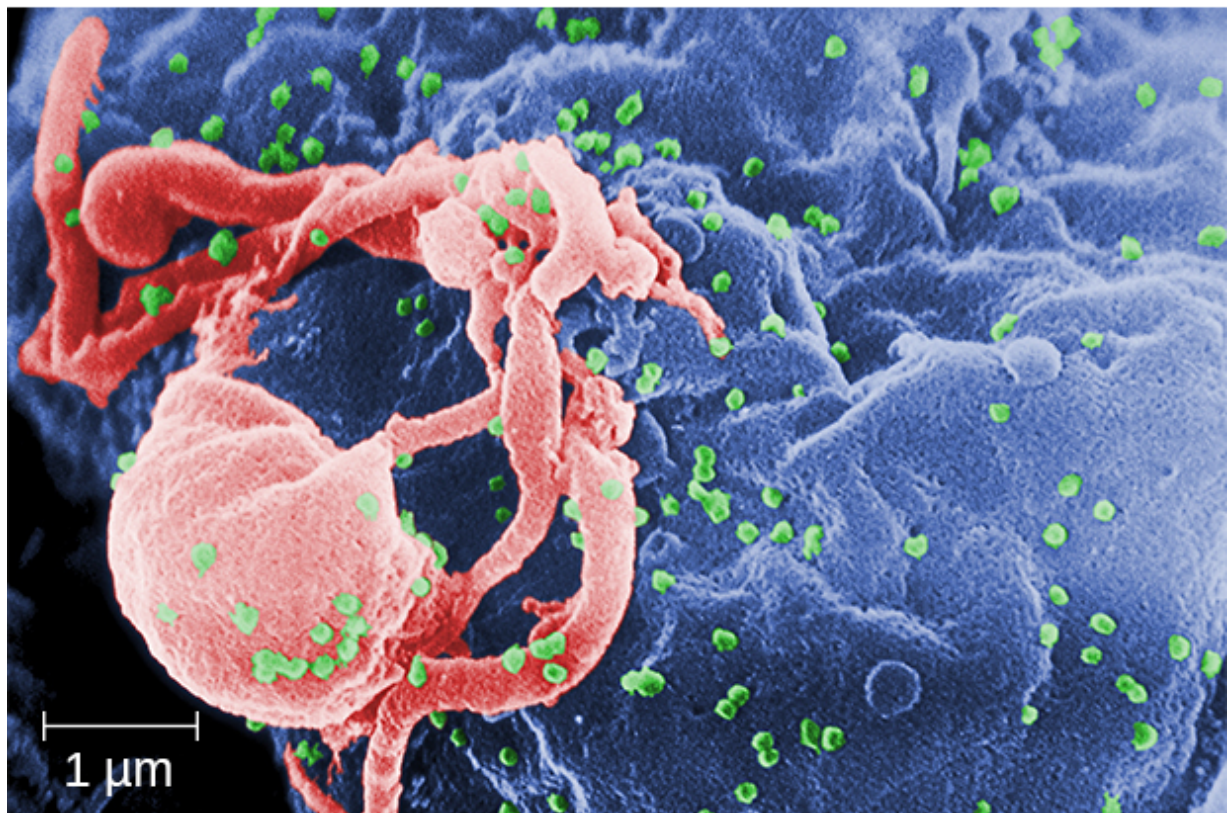
[\[footnote\]](#) Sub-Saharan Africa also accounts for two-thirds of the global total of new HIV infections ([\[link\]](#)).[\[footnote\]](#)

World Health Organization. “HIV/AIDS: Fact Sheet.”

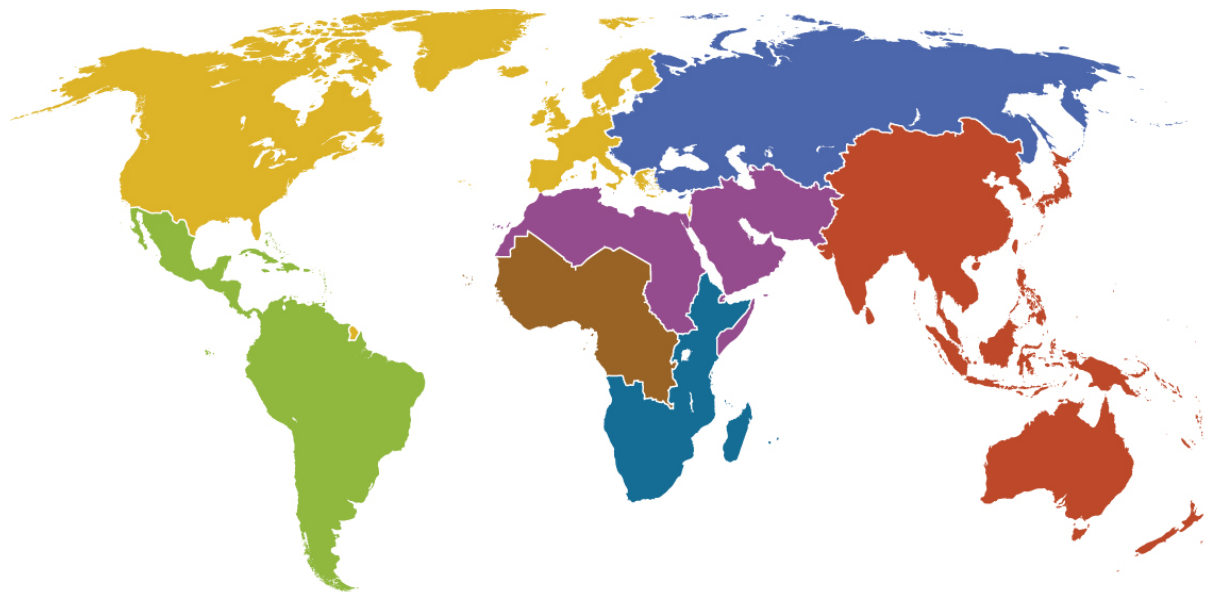
2016.<http://www.who.int/mediacentre/factsheets/fs360/en/>. Accessed July 28, 2016.

ibid.

HIV-1



This micrograph shows HIV particles (green) budding from a lymphocyte (top right). (credit: modification of work by Centers for Disease Control and Prevention)



Global prevalence in 2015		= 0.8%	
■ Middle East and North Africa	= 0.1%	■ Latin America and the Caribbean	= 0.5%
■ Asia and the Pacific	= 0.2%	■ Eastern Europe and Central Asia	= 0.9%
■ Western and Central Europe and North America	= 0.3%	■ West and Central Africa	= 2.2%
		■ East and Southern Africa	= 7.1%

This map shows the prevalence of HIV worldwide in 2015 among adults ages 15–49 years.

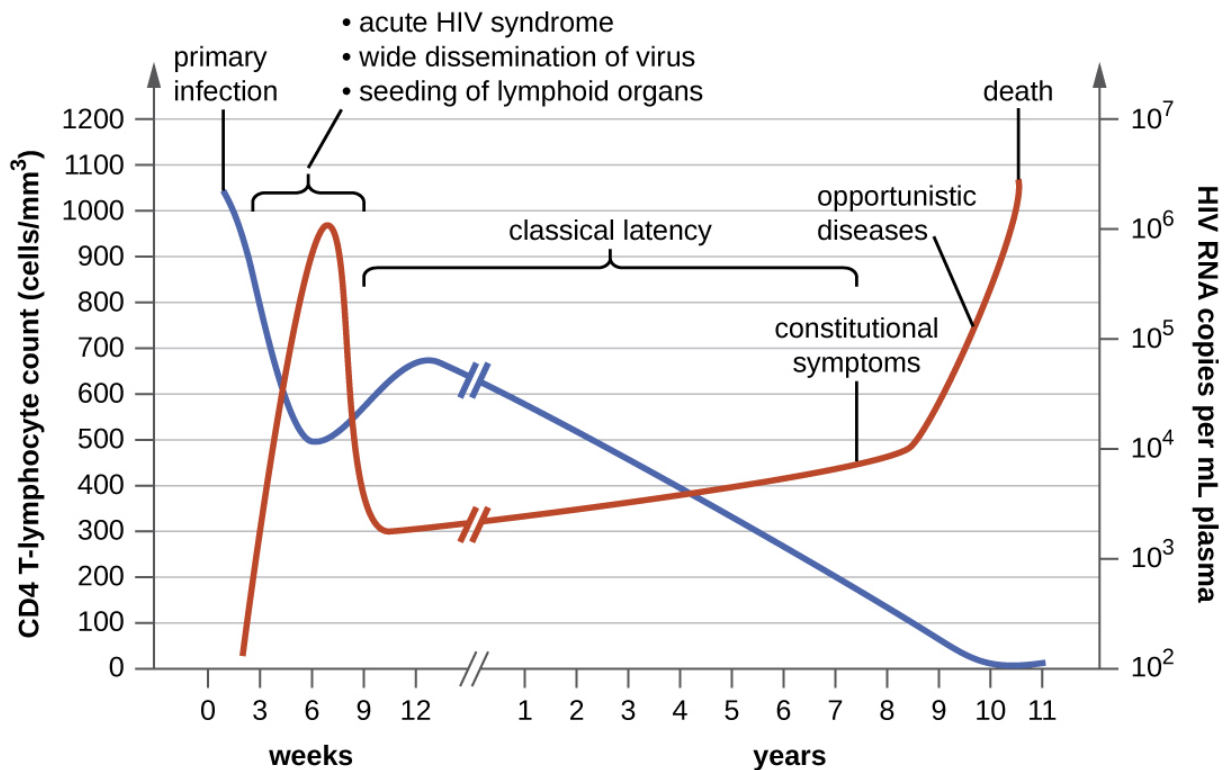
HIV is spread through direct contact with body fluids. Casual contact and insect vectors are not sufficient for disease transmission; common modes of transmission include sexual contact and sharing of needles by intravenous (IV) drug users. It generally takes many years before the effects of an HIV infection are detected. HIV infections are not dormant during this period: virions are continually produced, and the immune system continually

attempts to clear the viral infection, while the virus persistently infects additional CD4 T cells. Over time, the CD4 T-cell population is devastated, ultimately leading to AIDS.

When people are infected with HIV, their disease progresses through three stages based on CD4 T-cell counts and the presence of clinical symptoms ([link](#)).

- **Stage 1: Acute HIV infection.** Two to 4 weeks after infection with HIV, patients may experience a flu-like illness, which can last for a few weeks. Patients with acute HIV infection have more than 500 cells/ μ L CD4 T cells and a large amount of virus in their blood. Patients are very contagious during this stage. To confirm acute infection, either a fourth-generation antibody-antigen test or a nucleic acid test (NAT) must be performed.
- **Stage 2: Clinical latency.** During this period, HIV enters a period of dormancy. Patients have between 200 and 499 cells/ μ L CD4 T cells; HIV is still active but reproduces at low levels, and patients may not experience any symptoms of illness. For patients who are not taking medicine to treat HIV, this period can last a decade or longer. For patients receiving antiretroviral therapy, the stage may last for several decades, and those with low levels of the virus in their blood are much less likely to transmit HIV than those who are not virally suppressed. Near the end of the latent stage, the patient's viral load starts to increase and the CD4 T-cell count begins to decrease, leading to the development of symptoms and increased susceptibility to opportunistic infections.
- **Stage 3: Acquired immunodeficiency syndrome (AIDS).** Patients are diagnosed with AIDS when their CD4 T-cell count drops below 200 cells/ μ L or when they develop certain opportunistic illnesses. During this stage, the immune system becomes severely damaged by HIV. Common symptoms of AIDS include chills, fever, sweats, swollen lymph glands, weakness, and weight loss; in addition, patients often develop rare cancers such as Kaposi's sarcoma and opportunistic infections such as *Pneumocystis pneumonia*, tuberculosis, cryptosporidiosis, and toxoplasmosis. This is a fatal progression that, in the terminal stages, includes wasting syndrome and dementia

complex. Patients with AIDS have a high viral load and are highly infectious; they typically survive about 3 years without treatment.



This graph shows the clinical progression of CD4 T cells (blue line), clinical symptoms, and viral RNA (red line) during an HIV infection.
(credit: modification of work by Kogan M, and Rappaport J)

The initial diagnosis of HIV is performed using a serological test for antibody production against the pathogen. Positive test results are confirmed by Western blot or PCR tests. It can take weeks or months for the body to produce antibodies in response to an infection. There are fourth-generation tests that detect HIV antibodies and HIV antigens that are present even before the body begins producing antibodies. Nucleic acid tests (NATs) are a third type of test that is relatively expensive and uncommon; NAT can detect HIV in blood and determine the viral load.

As a consequence of provirus formation, it is currently not possible to eliminate HIV from an infected patient's body. Elimination by specific antibodies is ineffective because the virus mutates rapidly—a result of the error-prone reverse transcriptase and the inability to correct errors. Antiviral treatments, however, can greatly extend life expectancy. To combat the problem of drug resistance, combinations of antiretroviral drugs called antiretroviral therapy (ART), sometimes called highly active ART or combined ART, are used. There are several different targets for antiviral drug action (and a growing list of drugs for each of these targets). One class of drugs inhibits HIV entry; other classes inhibit reverse transcriptase by blocking viral RNA-dependent and DNA-dependent DNA polymerase activity; and still others inhibit one of the three HIV enzymes needed to replicate inside human cells.

Note:

- Why is it not yet possible to cure HIV infections?

Note:

HIV, AIDS, and Education

When the first outbreaks of AIDS in the US occurred in the early 1980s, very little was known about the disease or its origins. Erroneously, the disease quickly became stigmatized as one associated with what became identified as at-risk behaviors such as sexual promiscuity, homosexuality, and IV drug use, even though mounting evidence indicated the disease was also contracted through transfusion of blood and blood products or by fetuses of infected mothers. In the mid-1980s, scientists elucidated the identity of the virus, its mode of transmission, and mechanisms of pathogenesis. Campaigns were undertaken to educate the public about how HIV spreads to stem infection rates and encourage behavioral changes that reduced the risk for infection. Approaches to this campaign, however, emphasized very different strategies. Some groups favored educational programs that emphasized sexual abstinence, monogamy, heterosexuality,

and “just say no to drugs.” Other groups placed an emphasis on “safe sex” in sex education programs and advocated social services programs that passed out free condoms to anyone, including sexually active minors, and provided needle exchange programs for IV drug users.

These are clear examples of the intersection between disease and cultural values. As a future health professional, what is your responsibility in terms of educating patients about behaviors that put them at risk for HIV or other diseases while possibly setting your own personal opinions aside? You will no doubt encounter patients whose cultural and moral values differ from your own. Is it ethical for you to promote your own moral agenda to your patients? How can you advocate for practical disease prevention while still respecting the personal views of your patients?

Note:

Viral Diseases of the Circulatory and Lymphatic Systems

Many viruses are able to cause systemic, difficult-to-treat infections because of their ability to replicate within the host. Some of the more common viruses that affect the circulatory system are summarized in [\[link\]](#).

Viral Diseases of the Circulatory and Lymphatic Systems					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
AIDS/HIV infection	Human immunodeficiency virus (HIV)	Flu-like symptoms during acute stage, followed by long period of clinical latency; final stage (AIDS) includes fever, weight loss, wasting syndrome, dementia, and opportunistic secondary infections leading to death	Contact with body fluids (e.g., sexual contact, use of contaminated needles)	Serological tests for antibodies and/or HIV antigens; nucleic acid test (NAT) for presence of virus	Antiretroviral therapy (ART) using various combinations of drugs
Burkitt lymphoma	Epstein-Barr virus (human herpesvirus-4 [HHV-4])	Rapid formation of malignant B-cell tumors, oral hairy leukoplakia; fatal if not promptly treated	Contact with body fluids (e.g., saliva, blood, semen); primarily affects patients immunocompromised by HIV or malaria	CT scans, tumor biopsy	Intensive alternating chemotherapy regimen
Chikungunya fever	Chikungunya virus	Fever, rash, joint pain	Transmitted between humans by <i>Aedes aegypti</i> and <i>A. albopictus</i> vectors	Viral culture, IFA, EIA, ELISA, PCR, RT-PCR	None
Cytomegalovirus infection	Cytomegalovirus (HHV-5)	Usually asymptomatic but may cause non-Epstein-Barr mononucleosis in adults; may cause developmental issues in developing fetus; in transplant recipients, may cause fever, transplant rejection, death	Contact with body fluids, blood transfusions, organ transplants; infected mothers can transmit virus to fetus transplacentally or to newborn in breastmilk, saliva	Histology, culture, EIA, IFA, PCR	Ganciclovir, valganciclovir, foscarnet, cidofovir
Dengue fever (breakbone fever)	Dengue fever viruses 1–4	Fever, headache, extreme bone and joint pain, abdominal pain, vomiting, hemorrhaging; can be fatal	Transmitted between humans by <i>A. aegypti</i> and <i>A. albopictus</i> vectors	Serologic testing, ELISA, and PCR	None
Ebola virus disease (EVD)	Ebola virus	Fever, headache, joint pain, diarrhea, vomiting, hemorrhaging in gastrointestinal tract, organ failure; often fatal	Contact with body fluids (e.g., blood, saliva, sweat, urine, feces, vomit); highly contagious	ELISA, IgM ELISA, PCR, virus isolation	None
Hantavirus pulmonary syndrome (HPS)	Hantavirus	Initial flu-like symptoms followed by pulmonary edema and hypotension leading to pneumonia and shock; can be fatal	Inhalation of dried feces, urine from infected mouse or rat	ELISA, Western blot, RIBA, RT-PCR	None
Hemorrhagic fever with renal syndrome	Hantavirus	Fever, headache, nausea, rash, or eye inflammation, followed by hemorrhaging and kidney failure; can be fatal	Inhalation of dried feces, urine from infected mouse or rat	ELISA, Western blot, RIBA, RT-PCR	None
Infectious mononucleosis	Epstein-Barr virus (HHV-4), cytomegalovirus (HHV-5)	Pharyngitis, fever, extreme fatigue; swelling of lymph nodes, spleen, and liver	Contact with body fluids (e.g., saliva, blood, semen)	Tests for antibodies to various EBV-associated antigens	None
Yellow fever	Yellow fever virus	Dizziness, fever, chills, headache, myalgia, nausea, vomiting, constipation, fatigue; moderate to severe cases may include jaundice, rash, mucosal hemorrhaging, seizures, shock, and death	From monkeys to humans or between humans via <i>Aedes</i> or <i>Haemagogus</i> mosquito vectors	Culture, serology, PCR	None for treatment; preventive vaccine available

Key Concepts and Summary

- Human herpesviruses such **Epstein-Barr virus** (HHV-4) and **cytomegalovirus** (HHV-5) are widely distributed. The former is associated with infectious mononucleosis and Burkitt lymphoma, and the latter can cause serious congenital infections as well as serious disease in immunocompromised adults.
- Arboviral diseases such as **yellow fever**, **dengue fever**, and **chikungunya fever** are characterized by high fevers and vascular damage that can often be fatal. **Ebola virus disease** is a highly contagious and often fatal infection spread through contact with bodily fluids.
- Although there is a vaccine available for yellow fever, treatments for patients with yellow fever, dengue, chikungunya fever, and Ebola virus disease are limited to supportive therapies.
- Patients infected with **human immunodeficiency virus (HIV)** progress through three stages of disease, culminating in **AIDS**. **Antiretroviral therapy (ART)** uses various combinations of drugs to suppress viral loads, extending the period of latency and reducing the likelihood of transmission.
- Vector control and animal reservoir control remain the best defenses against most viruses that cause diseases of the circulatory system.

Multiple Choice

Exercise:

Problem:

Which of the following viruses is most widespread in the human population?

- A. human immunodeficiency virus
- B. Ebola virus
- C. Epstein-Barr virus
- D. hantavirus

Solution:

C

Exercise:

Problem:

Which of these viruses is spread through mouse urine or feces?

- A. Epstein-Barr
- B. hantavirus
- C. human immunodeficiency virus
- D. cytomegalovirus

Solution:

B

Exercise:

Problem:

A patient at a clinic has tested positive for HIV. Her blood contained 700/ μ L CD4 T cells and she does not have any apparent illness. Her infection is in which stage?

- A. 1
- B. 2
- C. 3

Solution:

A

Fill in the Blank

Exercise:

Problem:

_____ is a cancer that forms in patients with HHV-4 and malaria coinfections.

Solution:

Burkitt lymphoma

Exercise:

Problem:

_____ are transmitted by vectors such as ticks or mosquitoes.

Solution:

Arboviruses

Exercise:

Problem: Infectious mononucleosis is caused by _____ infections.

Solution:

Epstein-Barr virus

Short Answer

Exercise:

Problem:

Describe the progression of an HIV infection over time with regard to the number of circulating viruses, host antibodies, and CD4 T cells.

Exercise:

Problem:

Describe the general types of diagnostic tests used to diagnose patients infected with HIV.

Exercise:

Problem:

Identify the general categories of drugs used in ART used to treat patients infected with HIV.

Critical Thinking

Exercise:

Problem:

Which is a bigger threat to the US population, Ebola or yellow fever? Why?

Parasitic Infections of the Circulatory and Lymphatic Systems

LEARNING OBJECTIVES

- Identify common parasites that cause infections of the circulatory and lymphatic systems
- Compare the major characteristics of specific parasitic diseases affecting the circulatory and lymphatic systems

Some protozoa and parasitic flukes are also capable of causing infections of the human circulatory system. Although these infections are rare in the US, they continue to cause widespread suffering in the developing world today. Fungal infections of the circulatory system are very rare. Therefore, they are not discussed in this chapter.

Malaria

Despite more than a century of intense research and clinical advancements, **malaria** remains one of the most important infectious diseases in the world today. Its widespread distribution places more than half of the world's population in jeopardy. In 2015, the WHO estimated there were about 214 million cases of malaria worldwide, resulting in about 438,000 deaths; about 88% of cases and 91% of deaths occurred in Africa.[\[footnote\]](#) Although malaria is not currently a major threat in the US, the possibility of its reintroduction is a concern. Malaria is caused by several protozoan parasites in the genus *Plasmodium*: *P. falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax*. *Plasmodium* primarily infect red blood cells and are transmitted through the bite of *Anopheles* mosquitoes.

World Health Organization. “World Malaria Report 2015: Summary.” 2015. <http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/>. Accessed July 28, 2016.

Currently, *P. falciparum* is the most common and most lethal cause of malaria, often called falciparum malaria. Falciparum malaria is widespread in highly populated regions of Africa and Asia, putting many people at risk for the most severe form of the disease.

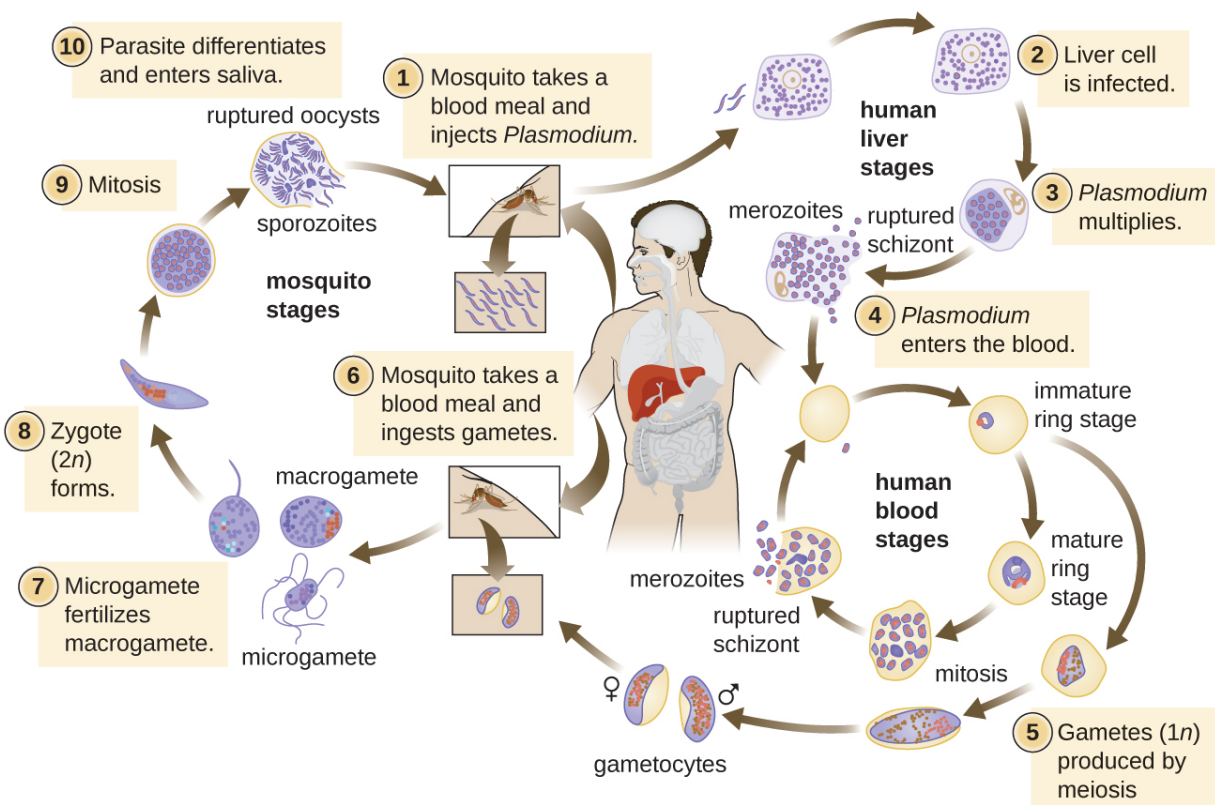
The classic signs and symptoms of malaria are cycles of extreme fever and chills. The sudden, violent symptoms of malaria start with malaise, abrupt chills, and fever (39–41° C [102.2–105.8 °F]), rapid and faint pulse, polyuria, headache, myalgia, nausea, and vomiting. After 2 to 6 hours of these symptoms, the fever falls, and profuse sweating occurs for 2 to 3 hours, followed by extreme fatigue. These symptoms are a result of *Plasmodium* emerging from red blood cells synchronously, leading to simultaneous rupture of a large number of red blood cells, resulting in damage to the spleen, liver, lymph nodes, and bone marrow. The organ damage resulting from hemolysis causes patients to develop sludge blood (i.e., blood in which the red blood cells agglutinate into clumps) that can lead to lack of oxygen, necrosis of blood vessels, organ failure, and death.

In established infections, malarial cycles of fever and chills typically occur every 2 days in the disease described as tertian malaria, which is caused by *P. vivax* and *P. ovale*. The cycles occur every 3 days in the disease described as quartan malaria, which is caused by *P. malariae*. These intervals may vary among cases.

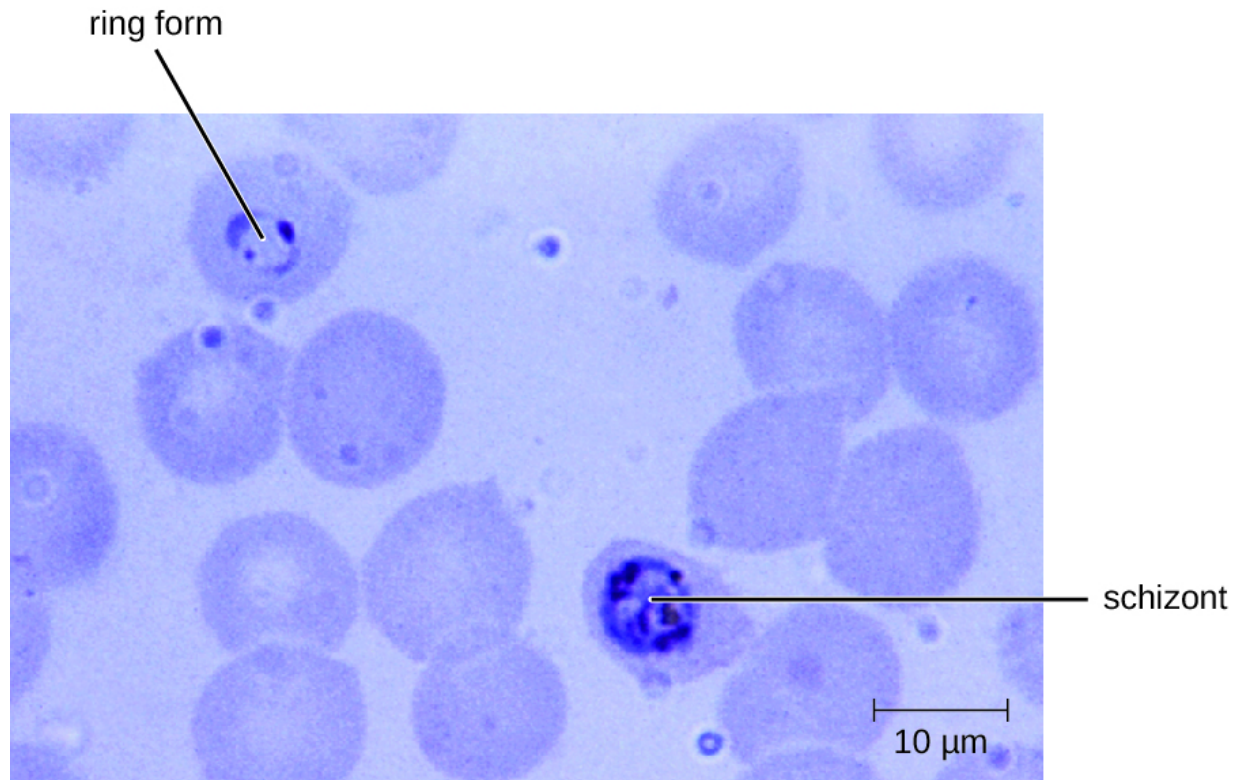
Plasmodium has a complex life cycle that includes several developmental stages alternately produced in mosquitoes and humans ([\[link\]](#)). When an infected mosquito takes a blood meal, sporozoites in the mosquito salivary gland are injected into the host's blood. These parasites circulate to the liver, where they develop into schizonts. The schizonts then undergo schizogony, resulting in the release of many merozoites at once. The merozoites move to the bloodstream and infect red blood cells. Inside red blood cells, merozoites develop into trophozoites that produce more merozoites. The synchronous release of merozoites from red blood cells in the evening leads to the symptoms of malaria.

In addition, some trophozoites alternatively develop into male and female gametocytes. The gametocytes are taken up when the mosquito takes a blood meal from an infected individual. Sexual sporogony occurs in the gut of the mosquito. The gametocytes fuse to form zygotes in the insect gut. The zygotes become motile and elongate into an ookinete. This form penetrates the midgut wall and develops into an oocyst. Finally, the oocyst releases new sporozoites that migrate to the mosquito salivary glands to complete the life cycle.

Diagnosis of malaria is by microscopic observation of developmental forms of *Plasmodium* in blood smears and rapid EIA assays that detect *Plasmodium* antigens or enzymes ([link](#)). Drugs such as chloroquine, atovaquone, artemether, and lumefantrine may be prescribed for both acute and prophylactic therapy, although some *Plasmodium* spp. have shown resistance to antimalarial drugs. Use of insecticides and insecticide-treated bed nets can limit the spread of malaria. Despite efforts to develop a vaccine for malaria, none is currently available.



The life cycle of *Plasmodium*. (credit: modification of work by Centers for Disease Control and Prevention)



A blood smear (human blood stage) shows an early trophozoite in a delicate ring form (upper left) and an early stage schizont form (center) of *Plasmodium falciparum* from a patient with malaria. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

Visit this [site](#) to learn how the parasite *Plasmodium* infects red blood cells.

The Nothing But Nets campaign, an initiative of the United Nations Foundation, has partnered with the Bill and Melinda Gates Foundation to make mosquito bed nets available in developing countries in Africa. Visit their [website](#) to learn more about their efforts to prevent malaria.

Note:

- Why is malaria one of the most important infectious diseases?

Toxoplasmosis

The disease **toxoplasmosis** is caused by the protozoan *Toxoplasma gondii*. *T. gondii* is found in a wide variety of birds and mammals, [\[footnote\]](#) and human infections are common. The Centers for Disease Control and Prevention (CDC) estimates that 22.5% of the population 12 years and older has been infected with *T. gondii*; but immunocompetent individuals are typically asymptomatic, however. [\[footnote\]](#) Domestic cats are the only known definitive hosts for the sexual stages of *T. gondii* and, thus, are the main reservoirs of infection. Infected cats shed *T. gondii* oocysts in their feces, and these oocysts typically spread to humans through contact with fecal matter on cats' bodies, in litter boxes, or in garden beds where outdoor cats defecate.

A.M. Tenter et al.. “*Toxoplasma gondii*: From Animals to Humans.” *International Journal for Parasitology* 30 no. 12-13 (2000):1217–1258.
Centers for Disease Control and Prevention. “Parasites - Toxoplasmosis (Toxoplasma Infection). Epidemiology & Risk Factors.” 2015
<http://www.cdc.gov/parasites/toxoplasmosis/epi.html>. Accessed July 28, 2016.

T. gondii has a complex life cycle that involves multiple hosts. The *T. gondii* life cycle begins when unsporulated oocysts are shed in the cat's feces. These oocysts take 1–5 days to sporulate in the environment and become infective. Intermediate hosts in nature include birds and rodents,

which become infected after ingesting soil, water, or plant material contaminated with the infective oocysts. Once ingested, the oocysts transform into tachyzoites that localize in the bird or rodent neural and muscle tissue, where they develop into tissue cysts. Cats may become infected after consuming birds and rodents harboring tissue cysts. Cats and other animals may also become infected directly by ingestion of sporulated oocysts in the environment. Interestingly, *Toxoplasma* infection appears to be able to modify the host's behavior. Mice infected by *Toxoplasma* lose their fear of cat pheromones. As a result, they become easier prey for cats, facilitating the transmission of the parasite to the cat definitive host[\[footnote\]](#) ([link](#)).

J. Flegr. "Effects of *Toxoplasma* on Human Behavior." *Schizophrenia Bulletin* 33, no. 3 (2007):757–760.

Toxoplasma infections in humans are extremely common, but most infected people are asymptomatic or have subclinical symptoms. Some studies suggest that the parasite may be able to influence the personality and psychomotor performance of infected humans, similar to the way it modifies behavior in other mammals.[\[footnote\]](#) When symptoms do occur, they tend to be mild and similar to those of mononucleosis. However, asymptomatic toxoplasmosis can become problematic in certain situations. Cysts can lodge in a variety of human tissues and lie dormant for years. Reactivation of these quiescent infections can occur in immunocompromised patients following transplantation, cancer therapy, or the development of an immune disorder such as AIDS. In patients with AIDS who have toxoplasmosis, the immune system cannot combat the growth of *T. gondii* in body tissues; as a result, these cysts can cause encephalitis, retinitis, pneumonitis, cognitive disorders, and seizures that can eventually be fatal.

Ibid

Toxoplasmosis can also pose a risk during pregnancy because tachyzoites can cross the placenta and cause serious infections in the developing fetus. The extent of fetal damage resulting from toxoplasmosis depends on the severity of maternal disease, the damage to the placenta, the gestational age of the fetus when infected, and the virulence of the organism. Congenital toxoplasmosis often leads to fetal loss or premature birth and can result in

damage to the central nervous system, manifesting as mental retardation, deafness, or blindness. Consequently, pregnant women are advised by the CDC to take particular care in preparing meat, gardening, and caring for pet cats.[\[footnote\]](#) Diagnosis of toxoplasmosis infection during pregnancy is usually achieved by serology including TORCH testing (the “T” in TORCH stands for toxoplasmosis). Diagnosis of congenital infections can also be achieved by detecting *T. gondii* DNA in amniotic fluid, using molecular methods such as PCR.

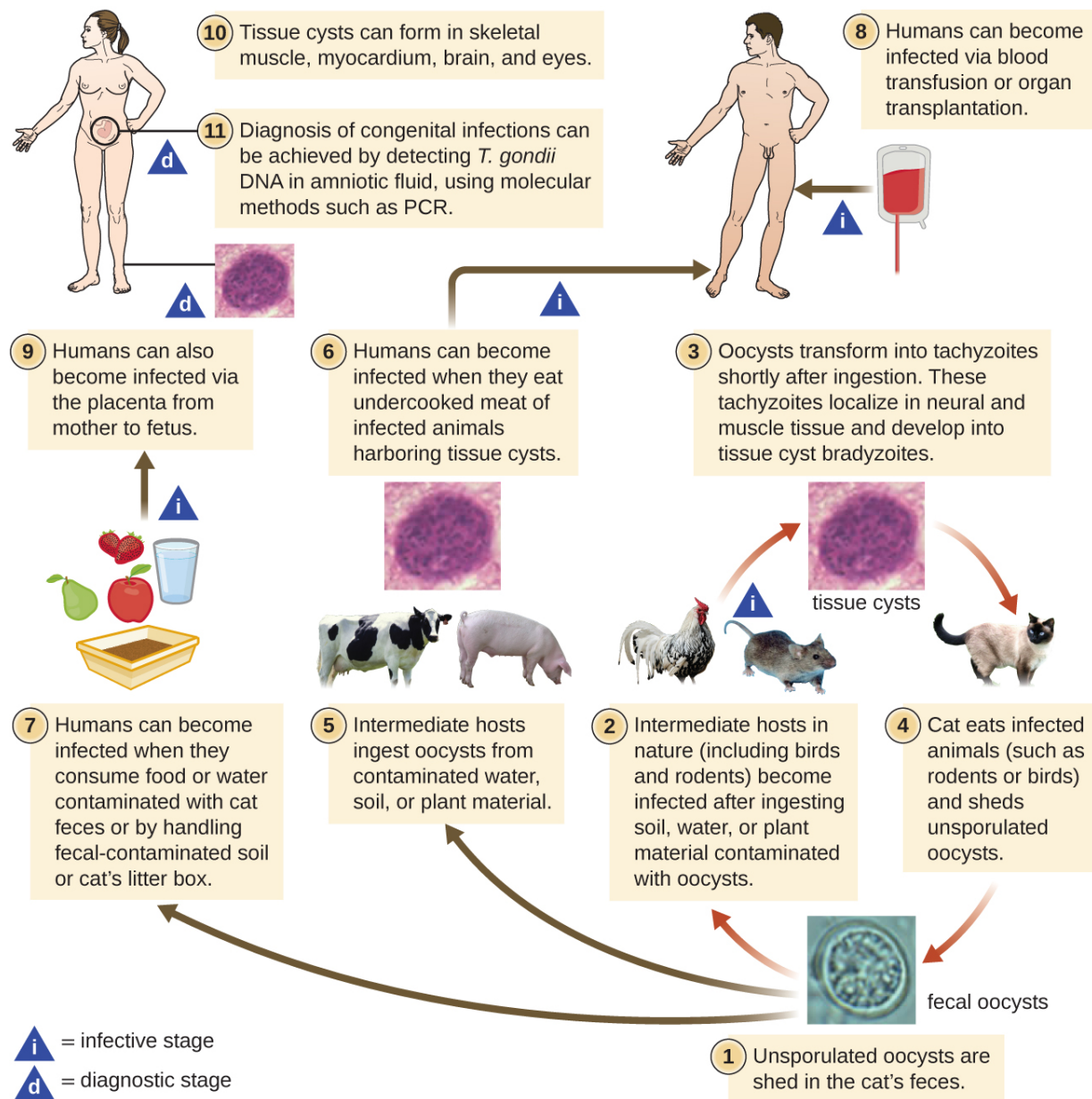
Centers for Disease Control and Prevention. “Parasites - Toxoplasmosis (Toxoplasma infection). Toxoplasmosis Frequently Asked Questions (FAQs).” 2013.

http://www.cdc.gov/parasites/toxoplasmosis/gen_info/faqs.html. Accessed July 28, 2016.

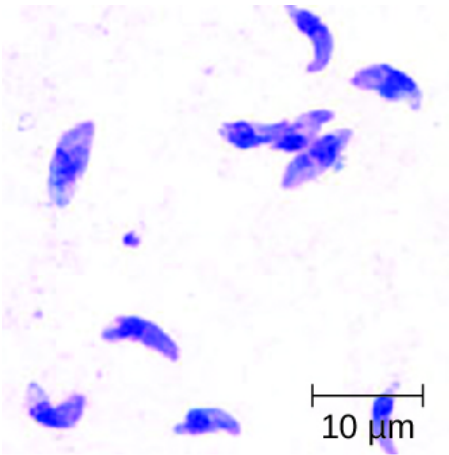
In adults, diagnosis of toxoplasmosis can include observation of tissue cysts in tissue specimens. Tissue cysts may be observed in Giemsa- or Wright-stained biopsy specimens, and CT, magnetic resonance imaging, and lumbar puncture can also be used to confirm infection ([\[link\]](#)).

Preventing infection is the best first-line defense against toxoplasmosis. Preventive measures include washing hands thoroughly after handling raw meat, soil, or cat litter, and avoiding consumption of vegetables possibly contaminated with cat feces. All meat should be cooked to an internal temperature of 73.9–76.7 °C (165–170 °F).

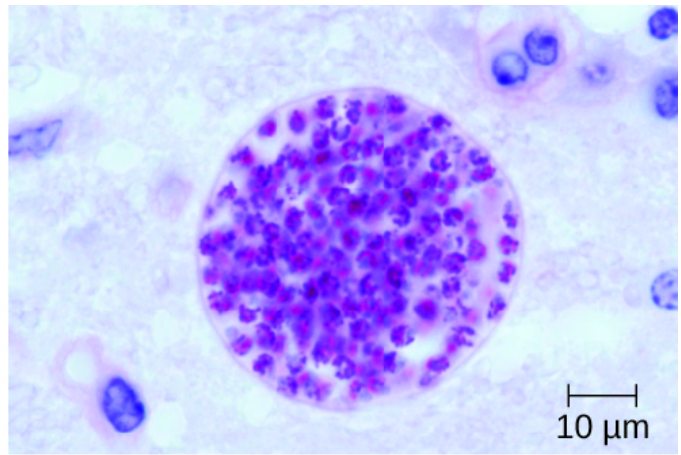
Most immunocompetent patients do not require clinical intervention for *Toxoplasma* infections. However, neonates, pregnant women, and immunocompromised patients can be treated with pyrimethamine and sulfadiazine—except during the first trimester of pregnancy, because these drugs can cause birth defects. Spiramycin has been used safely to reduce transmission in pregnant women with primary infection during the first trimester because it does not cross the placenta.



The infectious cycle of *Toxoplasma gondii*. (credit: “diagram”: modification of work by Centers for Disease Control and Prevention; credit “cat”: modification of work by “KaCey97078”/Flickr)



(a)



(b)

(a) Giemsa-stained *Toxoplasma gondii* tachyzoites from a smear of peritoneal fluid obtained from a mouse inoculated with *T. gondii*. Tachyzoites are typically crescent shaped with a prominent, centrally placed nucleus. (b) Microscopic cyst containing *T. gondii* from mouse brain tissue. Thousands of resting parasites (stained red) are contained in a thin parasite cyst wall. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by USDA)

Note:

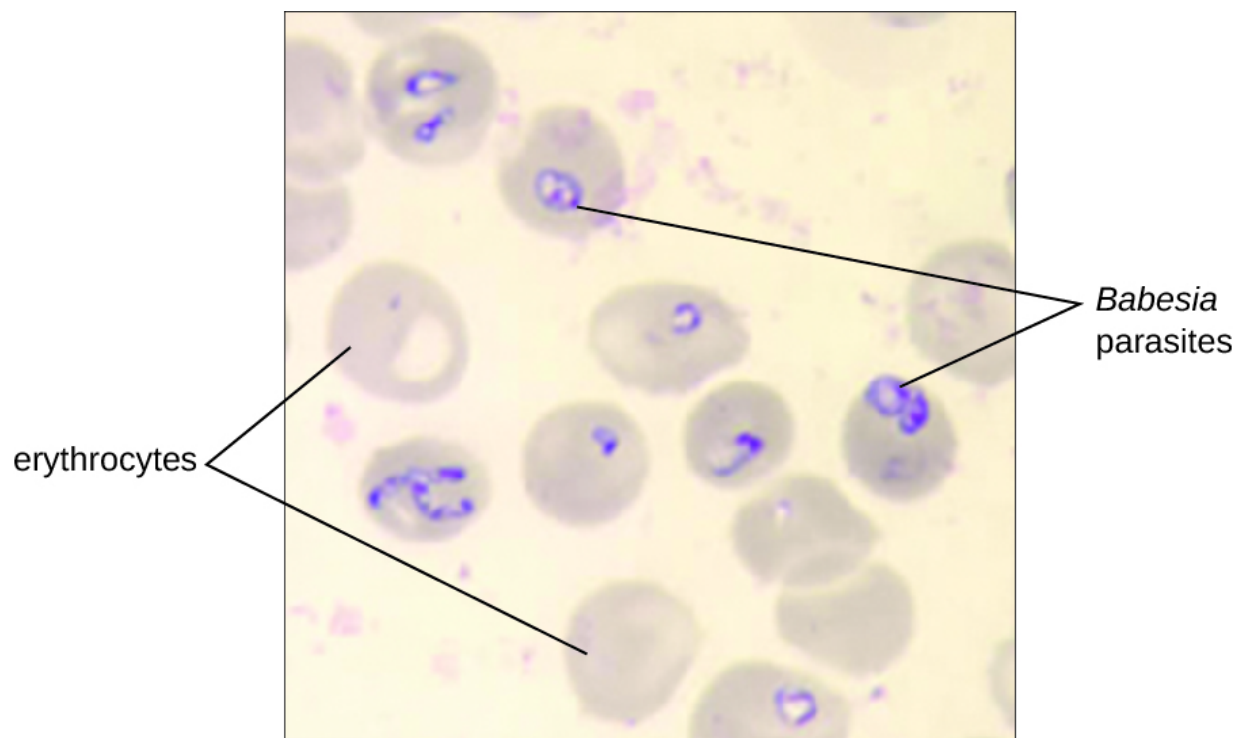
- How does *T. gondii* infect humans?

Babesiosis

Babesiosis is a rare zoonotic infectious disease caused by *Babesia* spp. These parasitic protozoans infect various wild and domestic animals and can be transmitted to humans by black-legged *Ixodes* ticks. In humans,

Babesia infect red blood cells and replicate inside the cell until it ruptures. The *Babesia* released from the ruptured red blood cell continue the growth cycle by invading other red blood cells. Patients may be asymptomatic, but those who do have symptoms often initially experience malaise, fatigue, chills, fever, headache, myalgia, and arthralgia. In rare cases, particularly in asplenic (absence of the spleen) patients, the elderly, and patients with AIDS, **babesiosis** may resemble falciparum malaria, with high fever, hemolytic anemia, hemoglobinuria (hemoglobin or blood in urine), jaundice, and renal failure, and the infection can be fatal. Previously acquired asymptomatic *Babesia* infection may become symptomatic if a splenectomy is performed.

Diagnosis is based mainly on the microscopic observation of parasites in blood smears ([\[link\]](#)). Serologic and antibody detection by IFA can also be performed and PCR-based tests are available. Many people do not require clinical intervention for *Babesia* infections, however, serious infections can be cleared with a combination of atovaquone and azithromycin or a combination of clindamycin and quinine.



In this blood smear from a patient with babesiosis, *Babesia* parasites can be observed in the red blood cells. (credit: modification of work by Centers for Disease Control and Prevention)

Chagas Disease

Also called American trypanosomiasis, Chagas disease is a zoonosis classified as a neglected tropical disease (NTD). It is caused by the flagellated protozoan *Trypanosoma cruzi* and is most commonly transmitted to animals and people through the feces of triatomine bugs. The triatomine bug is nicknamed the kissing bug because it frequently bites humans on the face or around the eyes; the insect often defecates near the bite and the infected fecal matter may be rubbed into the bite wound by the bitten individual ([\[link\]](#)). The bite itself is painless and, initially, many people show no signs of the disease. Alternative modes of transmission include contaminated blood transfusions, organ transplants from infected donors, and congenital transmission from mother to fetus.

Chagas disease is endemic throughout much of Mexico, Central America, and South America, where, according to WHO, an estimated 6 million to 7 million people are infected. [\[footnote\]](#) Currently, Chagas disease is not endemic in the US, even though triatomine bugs are found in the southern half of the country.

World Health Organization. “Chagas disease (American trypanosomiasis). Fact Sheet.” 2016. <http://www.who.int/mediacentre/factsheets/fs340/en/>. Accessed July 29, 2016.

Triatomine bugs typically are active at night, when they take blood meals by biting the faces and lips of people or animals as they sleep and often defecate near the site of the bite. Infection occurs when the host rubs the feces into their eyes, mouth, the bite wound, or another break in the skin. The protozoan then enters the blood and invades tissues of the heart and central nervous system, as well as macrophages and monocytes. Nonhuman reservoirs of *T. cruzi* parasites include wild animals and domesticated

animals such as dogs and cats, which also act as reservoirs of the pathogen.

[\[footnote\]](#)

C.E. Reisenman et al. “Infection of Kissing Bugs With *Trypanosoma cruzi*, Tucson, Arizona, USA.” *Emerging Infectious Diseases* 16 no. 3 (2010):400–405.

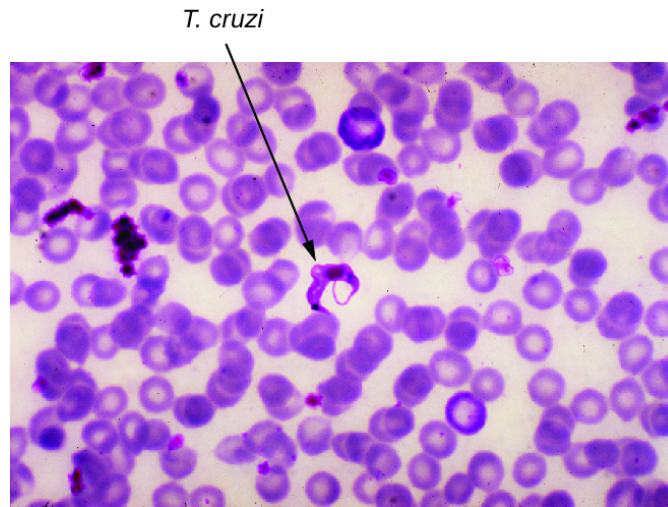
There are three phases of Chagas disease: acute, intermediate, and chronic. These phases can be either asymptomatic or life-threatening depending on the immunocompetence status of the patient.

In acute phase disease, symptoms include fever, headache, myalgia, rash, vomiting, diarrhea, and enlarged spleen, liver, and lymph nodes. In addition, a localized nodule called a chagoma may form at the portal of entry, and swelling of the eyelids or the side of the face, called Romaña's sign, may occur near the bite wound. Symptoms of the acute phase may resolve spontaneously, but if untreated, the infection can persist in tissues, causing irreversible damage to the heart or brain. In rare cases, young children may die of myocarditis or meningoencephalitis during the acute phase of Chagas disease.

Following the acute phase is a prolonged intermediate phase during which few or no parasites are found in the blood and most people are asymptomatic. Many patients will remain asymptomatic for life; however, decades after exposure, an estimated 20%–30% of infected people will develop chronic disease that can be debilitating and sometimes life threatening. In the chronic phase, patients may develop painful swelling of the colon, leading to severe twisting, constipation, and bowel obstruction; painful swelling of the esophagus, leading to dysphagia and malnutrition; and flaccid cardiomegaly (enlargement of the heart), which can lead to heart failure and sudden death.

Diagnosis can be confirmed through several different tests, including direct microscopic observation of trypanosomes in the blood, IFA, EIAs, PCR, and culturing in artificial media. In endemic regions, xenodiagnoses may be used; this method involves allowing uninfected kissing bugs to feed on the patient and then examining their feces for the presence of *T. cruzi*.

The medications nifurtimox and benznidazole are effective treatments during the acute phase of Chagas disease. The efficacy of these drugs is much lower when the disease is in the chronic phase. Avoiding exposure to the pathogen through vector control is the most effective method of limiting this disease.



(a)



(b)

(a) *Trypanosoma cruzi* protozoan in a blood smear from a patient with Chagas disease. (b) The triatomine bug (also known as the kissing bug or assassin bug) is the vector of Chagas disease. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Erwin Huebner)

Note:

- How do kissing bugs infect humans with *Trypanosoma cruzi*?

Leishmaniasis

Although it is classified as an NTD, **leishmaniasis** is relatively widespread in tropical and subtropical regions, affecting people in more than 90 countries. It is caused by approximately 20 different species of *Leishmania*, protozoan parasites that are transmitted by sand fly vectors such as *Phlebotomus* spp. and *Lutzomyia* spp. Dogs, cats, sheep, horses, cattle, rodents, and humans can all serve as reservoirs.

The *Leishmania* protozoan is phagocytosed by macrophages but uses virulence factors to avoid destruction within the phagolysosome. The virulence factors inhibit the phagolysosome enzymes that would otherwise destroy the parasite. The parasite reproduces within the macrophage, lyses it, and the progeny infect new macrophages (see [Micro Connections: When Phagocytosis Fails](#)).

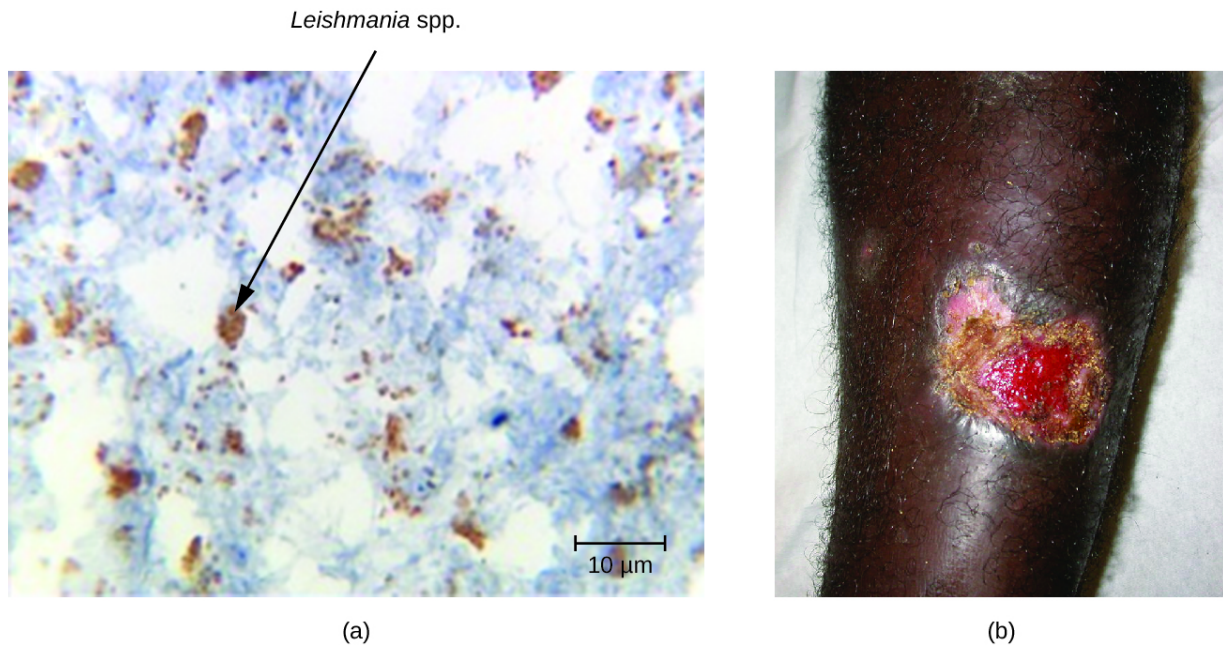
The three major clinical forms of leishmaniasis are cutaneous (oriental sore, Delhi boil, Aleppo boil), visceral (kala-azar, Dumdum fever), and mucosal (espundia). The most common form of disease is cutaneous leishmaniasis, which is characterized by the formation of sores at the site of the insect bite that may start out as papules or nodules before becoming large ulcers ([link](#)).

It may take visceral leishmaniasis months and sometimes years to develop, leading to enlargement of the lymph nodes, liver, spleen, and bone marrow. The damage to these body sites triggers fever, weight loss, and swelling of the spleen and liver. It also causes a decrease in the number of red blood cells (anemia), white blood cells (leukopenia), and platelets (thrombocytopenia), causing the patient to become immunocompromised and more susceptible to fatal infections of the lungs and gastrointestinal tract.

The mucosal form of leishmaniasis is one of the less common forms of the disease. It causes a lesion similar to the cutaneous form but mucosal leishmaniasis is associated with mucous membranes of the mouth, nares, or pharynx, and can be destructive and disfiguring. Mucosal leishmaniasis occurs less frequently when the original cutaneous (skin) infection is promptly treated.

Definitive diagnosis of leishmaniasis is made by visualizing organisms in Giemsa-stained smears, by isolating *Leishmania* protozoans in cultures, or by PCR-based assays of aspirates from infected tissues. Specific DNA probes or analysis of cultured parasites can help to distinguish *Leishmania* species that are causing simple cutaneous leishmaniasis from those capable of causing mucosal leishmaniasis.

Cutaneous leishmaniasis is usually not treated. The lesions will resolve after weeks (or several months), but may result in scarring. Recurrence rates are low for this disease. More serious infections can be treated with stibogluconate (antimony gluconate), amphotericin B, and miltefosine.



(a) A micrograph of a tissue sample from a patient with localized cutaneous leishmaniasis. Parasitic *Leishmania mexicana* (black arrow) are visible in and around the host cells. (b) Large skin ulcers are associated with cutaneous leishmaniasis. (credit a: modification of work by Fernández-Figueroa EA, Rangel-Escareño C, Espinosa-Mateos V, Carrillo-Sánchez K, Salaiza-Suazo N, Carrada-Figueroa G, March-Mifsut S, and Becker I; credit b: modification of work by Jean Fortunet)

Note:

- Compare the mucosal and cutaneous forms of leishmaniasis.

Schistosomiasis

Schistosomiasis (bilharzia) is an NTD caused by blood flukes in the genus *Schistosoma* that are native to the Caribbean, South America, Middle East, Asia, and Africa. Most human **schistosomiasis** cases are caused by *Schistosoma mansoni*, *S. haematobium*, or *S. japonicum*. *Schistosoma* are the only trematodes that invade through the skin; all other trematodes infect by ingestion. WHO estimates that at least 258 million people required preventive treatment for schistosomiasis in 2014.[\[footnote\]](#) World Health Organization. “Schistosomiasis. Fact Sheet.” 2016. <http://www.who.int/mediacentre/factsheets/fs115/en/>. Accessed July 29, 2016.

Infected human hosts shed *Schistosoma* eggs in urine and feces, which can contaminate freshwater habitats of snails that serve as intermediate hosts. The eggs hatch in the water, releasing miracidia, an intermediate growth stage of the *Schistosoma* that infect the snails. The miracidia mature and multiply inside the snails, transforming into cercariae that leave the snail and enter the water, where they can penetrate the skin of swimmers and bathers. The cercariae migrate through human tissue and enter the bloodstream, where they mature into adult male and female worms that mate and release fertilized eggs. The eggs travel through the bloodstream and penetrate various body sites, including the bladder or intestine, from which they are excreted in urine or stool to start the life cycle over again ([link](#)).

A few days after infection, patients may develop a rash or itchy skin associated with the site of cercariae penetration. Within 1–2 months of infection, symptoms may develop, including fever, chills, cough, and myalgia, as eggs that are not excreted circulate through the body. After years of infection, the eggs become lodged in tissues and trigger inflammation and scarring that can damage the liver, central nervous system, intestine, spleen, lungs, and bladder. This may cause abdominal pain, enlargement of the liver, blood in the urine or stool, and problems passing urine. Increased risk for bladder cancer is also associated with chronic *Schistosoma* infection. In addition, children who are repeatedly infected can develop malnutrition, anemia, and learning difficulties.

Diagnosis of schistosomiasis is made by the microscopic observation of eggs in feces or urine, intestine or bladder tissue specimens, or serologic tests. The drug praziquantel is effective for the treatment of all schistosome infections. Improving wastewater management and educating at-risk populations to limit exposure to contaminated water can help control the spread of the disease.

Cercarial Dermatitis

The cercaria of some species of *Schistosoma* can only transform into adult worms and complete their life cycle in animal hosts such as migratory birds and mammals. The cercaria of these worms are still capable of penetrating human skin, but they are unable to establish a productive infection in human tissue. Still, the presence of the cercaria in small blood vessels triggers an immune response, resulting in itchy raised bumps called **cercarial dermatitis** (also known as swimmer's itch or clam digger's itch). Although it is uncomfortable, cercarial dermatitis is typically self-limiting and rarely serious. Antihistamines and antipruritics can be used to limit inflammation and itching, respectively.

Note:

- How do schistosome infections in humans occur?

Note:

Common Eukaryotic Pathogens of the Human Circulatory System

Protozoan and helminthic infections are prevalent in the developing world. A few of the more important parasitic infections are summarized in [\[link\]](#).

Parasitic Diseases of the Circulatory and Lymphatic Systems					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Protozoa					
Babesiosis	<i>Babesia</i> spp.	Malaise, chills, fever, head-ache, myalgia, arthralgia	From animals to humans via <i>Ixodes</i> tick vectors	Blood smear, serology, IFA, and PCR	Atovaquone and azithromycin or clindamycin and quinine
Chagas disease	<i>Trypanosoma cruzi</i>	Fever, headache, body aches, swollen lymph nodes; potentially fatal	Between humans or from animal reservoirs via triatomine (kissing bug) vector	Blood smear, IFA, EIA, PCR, xenodiagnosis	Nifurtimox, benznidazole
Leishmaniasis	<i>Leishmania</i> spp.	Ulcer; enlargement of the lymph nodes, liver, spleen, and other organs	Between humans or from animal reservoirs via sand fly (<i>Phlebotomus</i> spp., <i>Lutzomyia</i> spp.) vectors	Blood smear, culture, PCR, DNA probe, biopsy	Stibogluconate, amphotericin B.
Malaria	<i>Plasmodium vivax</i> , <i>P. malariae</i> , <i>P. falciparum</i> , <i>P. ovale</i> , <i>P. knowlesi</i>	Extreme fever, chills, myalgia, nausea, and vomiting, possibly leading to organ failure and death	Between humans via <i>Anopheles</i> mosquito vectors	Blood smear, EIA	Chloroquine, atovaquone, artemether, and lumefantrine
Toxoplasmosis	<i>Toxoplasma gondii</i>	Tissue cysts; in pregnant women, birth defects or miscarriage	Contact with feces of infected cat; eating contaminated vegetables or undercooked meat of infected animal	Serological tests, direct detection of pathogen in tissue sections	Sulfadiazine, pyrimethamine, spiramycin
Helminths					
Schistosomiasis	<i>Schistosoma</i> spp.	Rash, fever, chills, myalgia; chronic inflammation and scarring of liver, spleen, and other organs where cysts develop	Snail hosts release cercaria into freshwater; cercaria burrow into skin of swimmers and bathers	Eggs in stool or urine, tissue biopsy, serological testing	Praziquantel

Note:**Resolution**

Despite continued antibiotic treatment and the removal of the venous catheter, Barbara's condition further declined. She began to show signs of shock and her blood pressure dropped to 77/50 mmHg. Anti-inflammatory drugs and drotrecogin- α were administered to combat sepsis. However, by the seventh day of hospitalization, Barbara experienced hepatic and renal failure and died.

Staphylococcus aureus most likely formed a biofilm on the surface of Barbara's catheter. From there, the bacteria were chronically shed into her circulation and produced the initial clinical symptoms. The chemotherapeutic therapies failed in large part because of the drug-resistant MRSA isolate. Virulence factors like leukocidin and hemolysins also interfered with her immune response. Barbara's ultimate decline may have been a consequence of the production of enterotoxins and toxic shock syndrome toxin (TSST), which can initiate toxic shock.

Venous catheters are common life-saving interventions for many patients requiring long-term administration of medication or fluids. However, they are also common sites of bloodstream infections. The World Health Organization estimates that there are up to 80,000 catheter-related bloodstream infections each year in the US, resulting in about 20,000 deaths. [\[footnote\]](#)

World Health Organization. "Patient Safety, Preventing Bloodstream Infections From Central Line Venous Catheters." 2016.

<http://www.who.int/patientsafety/implementation/bsi/en/>. Accessed July 29, 2016.

Go back to the [previous](#) Clinical Focus box.

Key Concepts and Summary

- **Malaria** is a protozoan parasite that remains an important cause of death primarily in the tropics. Several species in the genus *Plasmodium* are responsible for malaria and all are transmitted by *Anopheles* mosquitoes. *Plasmodium* infects and destroys human red blood cells, leading to organ damage, anemia, blood vessel necrosis,

and death. Malaria can be treated with various antimalarial drugs and prevented through vector control.

- **Toxoplasmosis** is a widespread protozoal infection that can cause serious infections in the immunocompromised and in developing fetuses. Domestic cats are the definitive host.
- Babesiosis is a generally asymptomatic infection of red blood cells that can cause malaria-like symptoms in elderly, immunocompromised, or asplenic patients.
- **Chagas disease** is a tropical disease transmitted by triatomine bugs. The trypanosome infects heart, neural tissues, monocytes, and phagocytes, often remaining latent for many years before causing serious and sometimes fatal damage to the digestive system and heart.
- **Leishmaniasis** is caused by the protozoan *Leishmania* and is transmitted by sand flies. Symptoms are generally mild, but serious cases may cause organ damage, anemia, and loss of immune competence.
- **Schistosomiasis** is caused by a fluke transmitted by snails. The fluke moves throughout the body in the blood stream and chronically infects various tissues, leading to organ damage.

Multiple Choice

Exercise:

Problem: Which of the following diseases is caused by a helminth?

- A. leishmaniasis
- B. malaria
- C. Chagas disease
- D. schistosomiasis

Solution:

D

Exercise:

Problem: Which of these is the most common form of leishmaniasis?

- A. cutaneous
- B. mucosal
- C. visceral
- D. intestinal

Solution:

A

Exercise:

Problem: Which of the following is a causative agent of malaria?

- A. *Trypanosoma cruzi*
- B. *Toxoplasma gondii*
- C. *Plasmodium falciparum*
- D. *Schistosoma mansoni*

Solution:

C

Exercise:

Problem:

Which of the following diseases does not involve an arthropod vector?

- A. schistosomiasis
- B. malaria
- C. Chagas disease
- D. babesiosis

Solution:

A

Fill in the Blank

Exercise:

Problem: The _____ mosquito is the biological vector for malaria.

Solution:

Anopheles

Exercise:

Problem: The kissing bug is the biological vector for _____.

Solution:

Chagas disease

Exercise:

Problem: Cercarial dermatitis is also known as _____.

Solution:

swimmer's itch

Short Answer

Exercise:

Problem:

Describe main cause of *Plasmodium falciparum* infection symptoms.

Exercise:

Problem:

Why should pregnant women avoid cleaning their cat's litter box or do so with protective gloves?

Critical Thinking

Exercise:

Problem:

What measures can be taken to reduce the likelihood of malaria reemerging in the US?

Introduction

class="introduction"

This dog is
exhibiting
the
restlessness
and
aggression
associated
with rabies,
a
neurological
disease that
frequently
affects
mammals
and can be
transmitted
to humans.

(credit:
modification
n of work
by the
Centers for
Disease
Control and
Prevention)



Few diseases inspire the kind of fear that rabies does. The name is derived from the Latin word for “madness” or “fury,” most likely because animals infected with rabies may behave with uncharacteristic rage and aggression. And while the thought of being attacked by a rabid animal is terrifying enough, the disease itself is even more frightful. Once symptoms appear, the disease is almost always fatal, even when treated.

Rabies is an example of a neurological disease caused by an acellular pathogen. The rabies virus enters nervous tissue shortly after transmission and makes its way to the central nervous system, where its presence leads to changes in behavior and motor function. Well-known symptoms associated with rabid animals include foaming at the mouth, hydrophobia (fear of water), and unusually aggressive behavior. Rabies claims tens of thousands of human lives worldwide, mainly in Africa and Asia. Most human cases result from dog bites, although many mammal species can become infected and transmit the disease. Human infection rates are low in the United States and many other countries as a result of control measures in animal populations. However, rabies is not the only disease with serious or fatal neurological effects. In this chapter, we examine the important microbial diseases of the nervous system.

Anatomy of the Nervous System

LEARNING OBJECTIVES

- Describe the major anatomical features of the nervous system
- Explain why there is no normal microbiota of the nervous system
- Explain how microorganisms overcome defenses of the nervous system to cause infection
- Identify and describe general symptoms associated with various infections of the nervous system

Note:

Part 1

David is a 35-year-old carpenter from New Jersey. A year ago, he was diagnosed with Crohn's disease, a chronic inflammatory bowel disease that has no known cause. He has been taking a prescription corticosteroid to manage the condition, and the drug has been highly effective in keeping his symptoms at bay. However, David recently fell ill and decided to visit his primary care physician. His symptoms included a fever, a persistent cough, and shortness of breath. His physician ordered a chest X-ray, which revealed consolidation of the right lung. The doctor prescribed a course of levofloxacin and told David to come back in a week if he did not feel better.

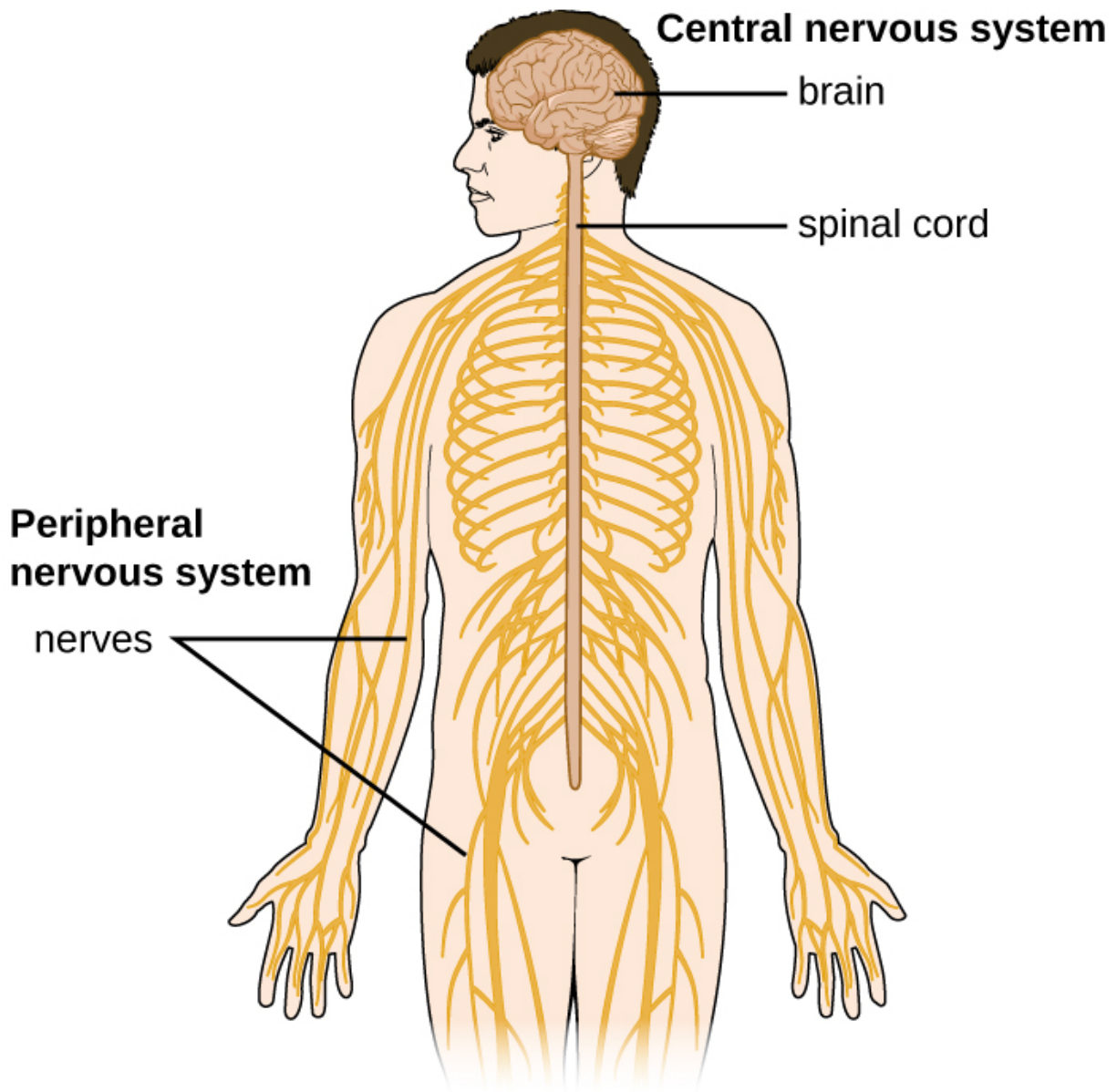
- What type of drug is levofloxacin?
- What type of microbes would this drug be effective against?
- What type of infection is consistent with David's symptoms?

Jump to the [next](#) Clinical Focus box.

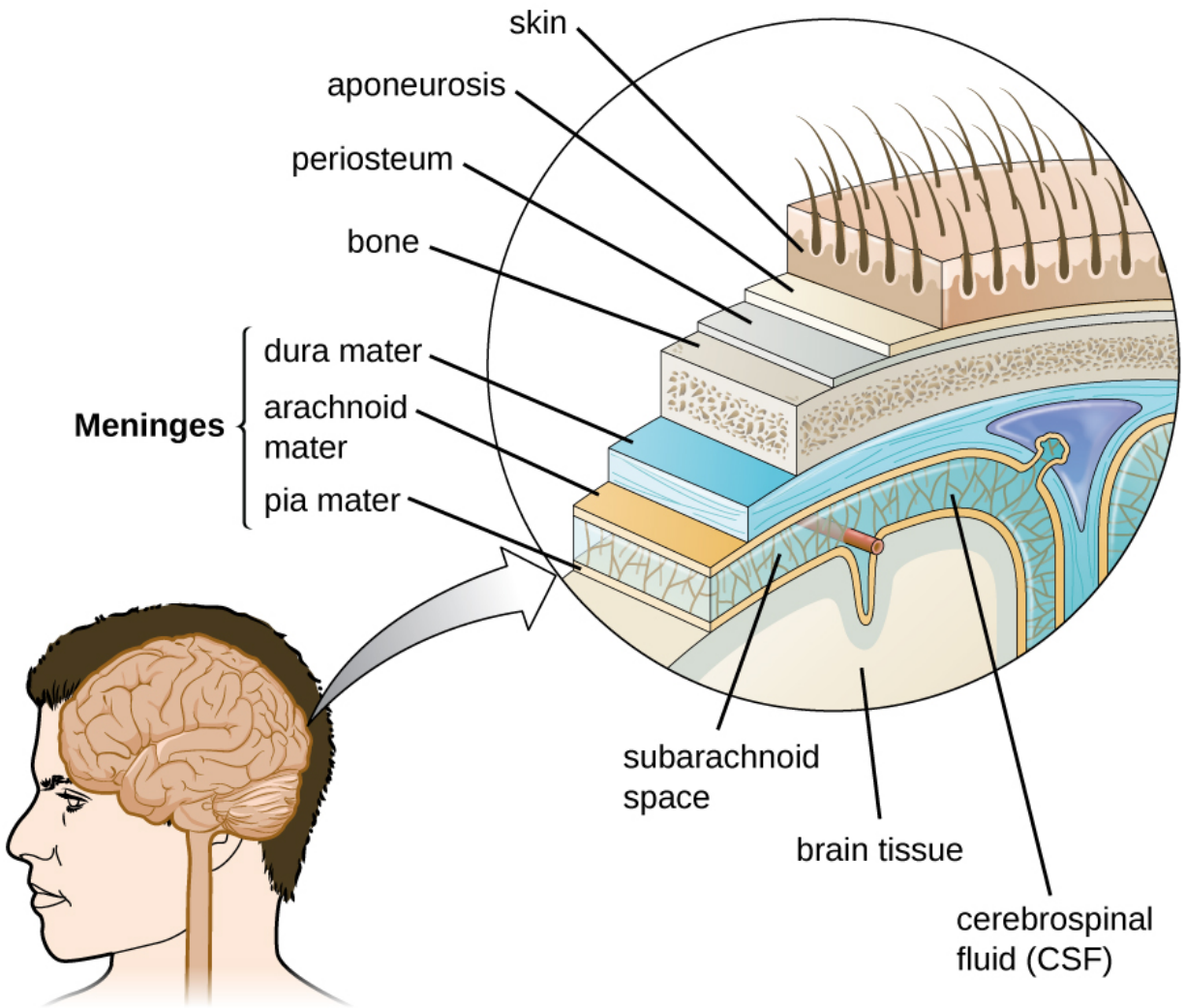
The human nervous system can be divided into two interacting subsystems: the **peripheral nervous system (PNS)** and the **central nervous system (CNS)**. The CNS consists of the brain and spinal cord. The peripheral nervous system is an extensive network of nerves connecting the CNS to the muscles and sensory structures. The relationship of these systems is illustrated in [\[link\]](#).

The Central Nervous System

The brain is the most complex and sensitive organ in the body. It is responsible for all functions of the body, including serving as the coordinating center for all sensations, mobility, emotions, and intellect. Protection for the brain is provided by the bones of the skull, which in turn are covered by the scalp, as shown in [\[link\]](#). The scalp is composed of an outer layer of skin, which is loosely attached to the aponeurosis, a flat, broad tendon layer that anchors the superficial layers of the skin. The periosteum, below the aponeurosis, firmly encases the bones of the skull and provides protection, nutrition to the bone, and the capacity for bone repair. Below the bony layer of the skull are three layers of membranes called **meninges** that surround the brain. The relative positions of these meninges are shown in [\[link\]](#). The meningeal layer closest to the bones of the skull is called the **dura mater** (literally meaning *tough mother*). Below the dura mater lies the **arachnoid mater** (literally *spider-like mother*). The innermost meningeal layer is a delicate membrane called the **pia mater** (literally *tender mother*). Unlike the other meningeal layers, the pia mater firmly adheres to the convoluted surface of the brain. Between the arachnoid mater and pia mater is the subarachnoid space. The subarachnoid space within this region is filled with **cerebrospinal fluid (CSF)**. This watery fluid is produced by cells of the choroid plexus—areas in each ventricle of the brain that consist of cuboidal epithelial cells surrounding dense capillary beds. The CSF serves to deliver nutrients and remove waste from neural tissues.



The essential components of the human nervous system are shown in this illustration. The central nervous system (CNS) consists of the brain and spinal cord. It connects to the peripheral nervous system (PNS), a network of nerves that extends throughout the body.



The layers of tissue surrounding the human brain include three meningeal membranes: the dura mater, arachnoid mater, and pia mater.
(credit: modification of work by National Institutes of Health)

The Blood-Brain Barrier

The tissues of the CNS have extra protection in that they are not exposed to blood or the immune system in the same way as other tissues. The blood vessels that supply the brain with nutrients and other chemical substances lie on top of the pia mater. The capillaries associated with these blood

vessels in the brain are less permeable than those in other locations in the body. The capillary endothelial cells form tight junctions that control the transfer of blood components to the brain. In addition, cranial capillaries have far fewer fenestra (pore-like structures that are sealed by a membrane) and pinocytotic vesicles than other capillaries. As a result, materials in the circulatory system have a very limited ability to interact with the CNS directly. This phenomenon is referred to as the blood-brain barrier.

The blood-brain barrier protects the cerebrospinal fluid from contamination, and can be quite effective at excluding potential microbial pathogens. As a consequence of these defenses, there is no normal microbiota in the cerebrospinal fluid. The blood-brain barrier also inhibits the movement of many drugs into the brain, particularly compounds that are not lipid soluble. This has profound ramifications for treatments involving infections of the CNS, because it is difficult for drugs to cross the blood-brain barrier to interact with pathogens that cause infections.

The spinal cord also has protective structures similar to those surrounding the brain. Within the bones of the vertebrae are meninges of dura mater (sometimes called the dural sheath), arachnoid mater, pia mater, and a blood-spinal cord barrier that controls the transfer of blood components from blood vessels associated with the spinal cord.

To cause an infection in the CNS, pathogens must successfully breach the blood-brain barrier or blood-spinal cord barrier. Various pathogens employ different virulence factors and mechanisms to achieve this, but they can generally be grouped into four categories: intercellular (also called paracellular), transcellular, leukocyte facilitated, and nonhematogenous. Intercellular entry involves the use of microbial virulence factors, toxins, or inflammation-mediated processes to pass between the cells of the blood-brain barrier. In transcellular entry, the pathogen passes through the cells of the blood-brain barrier using virulence factors that allow it to adhere to and trigger uptake by vacuole- or receptor-mediated mechanisms. Leukocyte-facilitated entry is a Trojan-horse mechanism that occurs when a pathogen infects peripheral blood leukocytes to directly enter the CNS. Nonhematogenous entry allows pathogens to enter the brain without encountering the blood-brain barrier; it occurs when pathogens travel along

either the olfactory or trigeminal cranial nerves that lead directly into the CNS.

Note:

View this [video](#) about the blood-brain barrier

Note:

- What is the primary function of the blood-brain barrier?

The Peripheral Nervous System

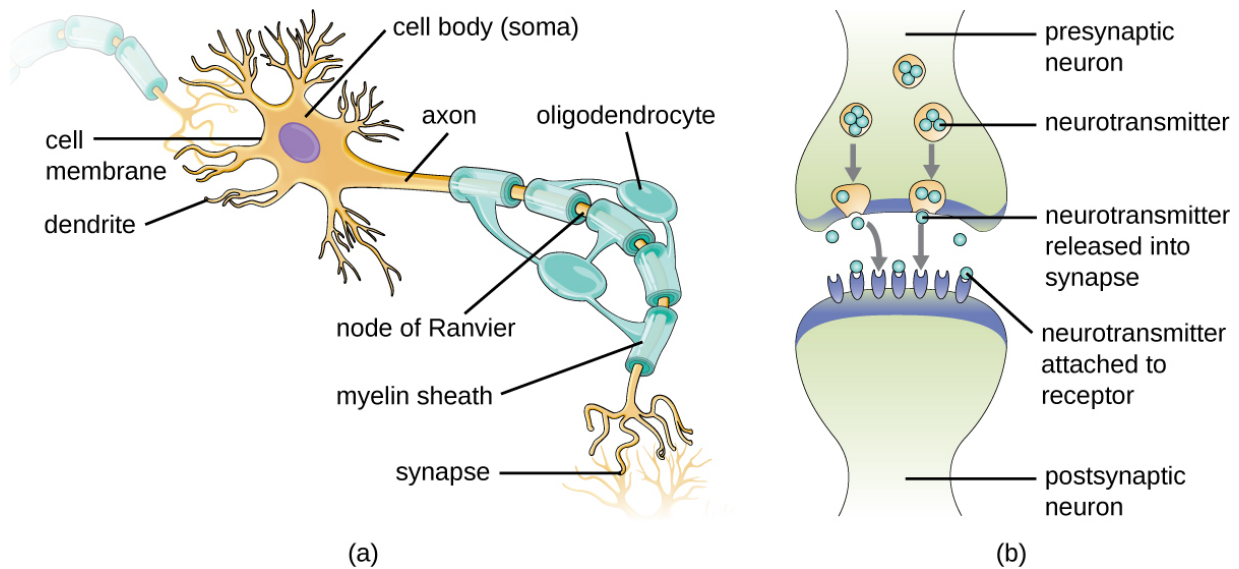
The PNS is formed of the nerves that connect organs, limbs, and other anatomic structures of the body to the brain and spinal cord. Unlike the brain and spinal cord, the PNS is not protected by bone, meninges, or a blood barrier, and, as a consequence, the nerves of the PNS are much more susceptible to injury and infection. Microbial damage to peripheral nerves can lead to tingling or numbness known as **neuropathy**. These symptoms can also be produced by trauma and noninfectious causes such as drugs or chronic diseases like diabetes.

The Cells of the Nervous System

Tissues of the PNS and CNS are formed of cells called **glial cells** (neuroglial cells) and **neurons** (nerve cells). Glial cells assist in the organization of neurons, provide a scaffold for some aspects of neuronal function, and aid in recovery from neural injury.

Neurons are specialized cells found throughout the nervous system that transmit signals through the nervous system using electrochemical processes. The basic structure of a neuron is shown in [\[link\]](#). The cell body

(or **soma**) is the metabolic center of the neuron and contains the nucleus and most of the cell's organelles. The many finely branched extensions from the soma are called **dendrites**. The soma also produces an elongated extension, called the **axon**, which is responsible for the transmission of electrochemical signals through elaborate ion transport processes. Axons of some types of neurons can extend up to one meter in length in the human body. To facilitate electrochemical signal transmission, some neurons have a **myelin sheath** surrounding the axon. Myelin, formed from the cell membranes of glial cells like the Schwann cells in the PNS and oligodendrocytes in the CNS, surrounds and insulates the axon, significantly increasing the speed of electrochemical signal transmission along the axon. The end of an axon forms numerous branches that end in bulbs called synaptic terminals. Neurons form junctions with other cells, such as another neuron, with which they exchange signals. The junctions, which are actually gaps between neurons, are referred to as **synapses**. At each synapse, there is a presynaptic neuron and a postsynaptic neuron (or other cell). The synaptic terminals of the axon of the presynaptic terminal form the synapse with the dendrites, soma, or sometimes the axon of the postsynaptic neuron, or a part of another type of cell such as a muscle cell. The synaptic terminals contain vesicles filled with chemicals called **neurotransmitters**. When the electrochemical signal moving down the axon reaches the synapse, the vesicles fuse with the membrane, and neurotransmitters are released, which diffuse across the synapse and bind to receptors on the membrane of the postsynaptic cell, potentially initiating a response in that cell. That response in the postsynaptic cell might include further propagation of an electrochemical signal to transmit information or contraction of a muscle fiber.



(a) A myelinated neuron is associated with oligodendrocytes. Oligodendrocytes are a type of glial cell that forms the myelin sheath in the CNS that insulates the axon so that electrochemical nerve impulses are transferred more efficiently. (b) A synapse consists of the axonal end of the presynaptic neuron (top) that releases neurotransmitters that cross the synaptic space (or cleft) and bind to receptors on dendrites of the postsynaptic neuron (bottom).

Note:

- What cells are associated with neurons, and what is their function?
- What is the structure and function of a synapse?

Meningitis and Encephalitis

Although the skull provides the brain with an excellent defense, it can also become problematic during infections. Any swelling of the brain or

meninges that results from inflammation can cause intracranial pressure, leading to severe damage of the brain tissues, which have limited space to expand within the inflexible bones of the skull. The term **meningitis** is used to describe an inflammation of the meninges. Typical symptoms can include severe headache, fever, photophobia (increased sensitivity to light), stiff neck, convulsions, and confusion. An inflammation of brain tissue is called **encephalitis**, and patients exhibit signs and symptoms similar to those of meningitis in addition to lethargy, seizures, and personality changes. When inflammation affects both the meninges and the brain tissue, the condition is called **meningoencephalitis**. All three forms of inflammation are serious and can lead to blindness, deafness, coma, and death.

Meningitis and encephalitis can be caused by many different types of microbial pathogens. However, these conditions can also arise from noninfectious causes such as head trauma, some cancers, and certain drugs that trigger inflammation. To determine whether the inflammation is caused by a pathogen, a lumbar puncture is performed to obtain a sample of CSF. If the CSF contains increased levels of white blood cells and abnormal glucose and protein levels, this indicates that the inflammation is a response to an infection.

Note:

- What are the two types of inflammation that can impact the CNS?
- Why do both forms of inflammation have such serious consequences?

Note:

Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) is a rare condition that can be preceded by a viral or bacterial infection that results in an autoimmune reaction against myelinated nerve cells. The destruction of the myelin sheath around these neurons results in a loss of sensation and function. The first symptoms of this condition are tingling and weakness in the affected tissues. The

symptoms intensify over a period of several weeks and can culminate in complete paralysis. Severe cases can be life-threatening. Infections by several different microbial pathogens, including *Campylobacter jejuni* (the most common risk factor), cytomegalovirus, Epstein-Barr virus, varicella-zoster virus, *Mycoplasma pneumoniae*, [\[footnote\]](#) and Zika virus [\[footnote\]](#) have been identified as triggers for GBS. Anti-myelin antibodies from patients with GBS have been demonstrated to also recognize *C. jejuni*. It is possible that cross-reactive antibodies, antibodies that react with similar antigenic sites on different proteins, might be formed during an infection and may lead to this autoimmune response.

Yuki, Nobuhiro and Hans-Peter Hartung, “Guillain–Barré Syndrome,” *New England Journal of Medicine* 366, no. 24 (2012): 2294-304.

Cao-Lormeau, Van-Mai, Alexandre Blake, Sandrine Mons, Stéphane Lastère, Claudine Roche, Jessica Vanhomwegen, Timothée Dub et al., “Guillain-Barré Syndrome Outbreak Associated with Zika Virus Infection in French Polynesia: A Case-Control Study,” *The Lancet* 387, no. 10027 (2016): 1531-9.

GBS is solely identified by the appearance of clinical symptoms. There are no other diagnostic tests available. Fortunately, most cases spontaneously resolve within a few months with few permanent effects, as there is no available vaccine. GBS can be treated by plasmapheresis. In this procedure, the patient’s plasma is filtered from their blood, removing autoantibodies.

Key Concepts and Summary

- The nervous system consists of two subsystems: the **central nervous system** and **peripheral nervous system**.
- The skull and three **meninges** (the **dura mater**, **arachnoid mater**, and **pia mater**) protect the brain.
- Tissues of the PNS and CNS are formed of cells called **glial cells** and **neurons**.
- Since the **blood-brain barrier** excludes most microbes, there is no normal microbiota in the CNS.

- Some pathogens have specific virulence factors that allow them to breach the blood-brain barrier. Inflammation of the brain or **meninges** caused by infection is called **encephalitis** or **meningitis**, respectively. These conditions can lead to blindness, deafness, coma, and death.

Multiple Choice

Exercise:

Problem:

What is the outermost membrane surrounding the brain called?

- a. pia mater
- b. arachnoid mater
- c. dura mater
- d. alma mater

Solution:

C

Exercise:

Problem: What term refers to an inflammation of brain tissues?

- a. encephalitis
- b. meningitis
- c. sinusitis
- d. meningoencephalitis

Solution:

A

Exercise:

Problem: Nerve cells form long projections called _____.

- a. soma
- b. axons
- c. dendrites
- d. synapses

Solution:

B

Exercise:

Problem:

Chemicals called _____ are stored in neurons and released when the cell is stimulated by a signal.

- a. toxins
- b. cytokines
- c. chemokines
- d. neurotransmitters

Solution:

D

Exercise:

Problem: The central nervous system is made up of

- a. sensory organs and muscles.
 - b. the brain and muscles.
 - c. the sensory organs and spinal cord.
 - d. the brain and spinal column.
-

Solution:

D

Matching**Exercise:****Problem:**

Match each strategy for microbial invasion of the CNS with its description.

___intercellular entry	A. pathogen gains entry by infecting peripheral white blood cells
___transcellular entry	B. pathogen bypasses the blood-brain barrier by travel along the olfactory or trigeminal cranial nerves
___leukocyte-facilitated entry	C. pathogen passes through the cells of the blood-brain barrier
___nonhematogenous entry	D. pathogen passes between the cells of the blood-brain barrier

Solution:

D, C, A, B

Fill in the Blank

Exercise:

Problem: The cell body of a neuron is called the _____.

Solution:

soma

Exercise:

Problem: A signal is transmitted down the _____ of a nerve cell.

Solution:

axon

Exercise:

Problem: The _____ is filled with cerebrospinal fluid.

Solution:

subarachnoid space

Exercise:

Problem:

The _____ prevents access of microbes in the blood from gaining access to the central nervous system.

Solution:

blood-brain barrier

Exercise:

Problem:

The _____ are a set of membranes that cover and protect the brain.

Solution:

meninges

Short Answer

Exercise:

Problem:

Briefly describe the defenses of the brain against trauma and infection.

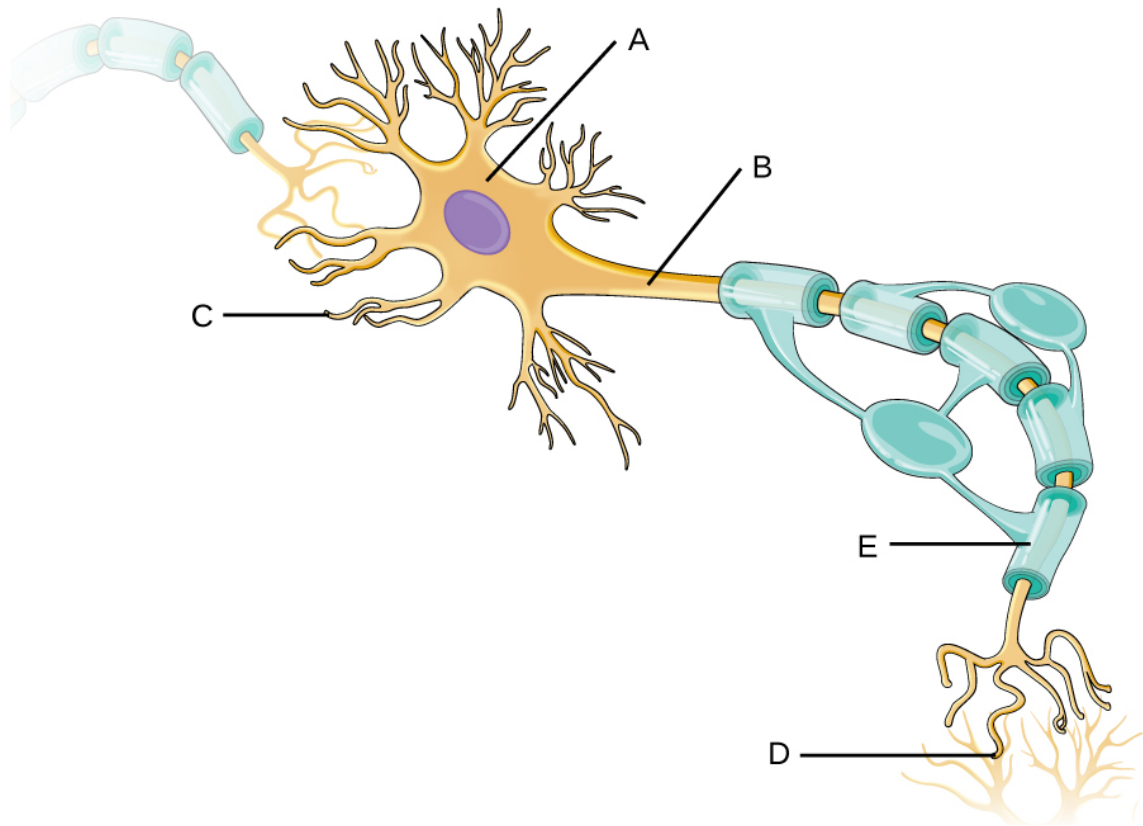
Exercise:

Problem: Describe how the blood-brain barrier is formed.

Exercise:

Problem:

Identify the type of cell shown, as well as the following structures:
axon, dendrite, myelin sheath, soma, and synapse.



Critical Thinking

Exercise:

Problem:

What important function does the blood-brain barrier serve? How might this barrier be problematic at times?

Bacterial Diseases of the Nervous System

LEARNING OBJECTIVES

- Identify the most common bacteria that can cause infections of the nervous system
- Compare the major characteristics of specific bacterial diseases affecting the nervous system

Bacterial infections that affect the nervous system are serious and can be life-threatening. Fortunately, there are only a few bacterial species commonly associated with neurological infections.

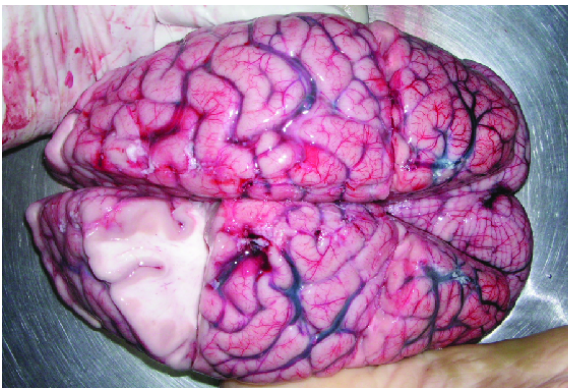
Bacterial Meningitis

Bacterial meningitis is one of the most serious forms of meningitis. Bacteria that cause meningitis often gain access to the CNS through the bloodstream after trauma or as a result of the action of bacterial toxins. Bacteria may also spread from structures in the upper respiratory tract, such as the oropharynx, nasopharynx, sinuses, and middle ear. Patients with head wounds or cochlear implants (an electronic device placed in the inner ear) are also at risk for developing meningitis.

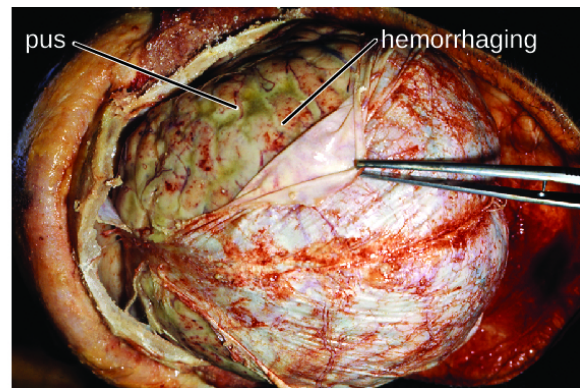
Many of the bacteria that can cause meningitis are commonly found in healthy people. The most common causes of non-neonatal bacterial meningitis are *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. All three of these bacterial pathogens are spread from person to person by respiratory secretions. Each can colonize and cross through the mucous membranes of the oropharynx and nasopharynx,

and enter the blood. Once in the blood, these pathogens can disseminate throughout the body and are capable of both establishing an infection and triggering inflammation in any body site, including the meninges ([\[link\]](#)). Without appropriate systemic antibacterial therapy, the case-fatality rate can be as high as 70%, and 20% of those survivors may be left with irreversible nerve damage or tissue destruction, resulting in hearing loss, neurologic disability, or loss of a limb. Mortality rates are much lower (as low as 15%) in populations where appropriate therapeutic drugs and preventive vaccines are available.[\[footnote\]](#)

Thigpen, Michael C., Cynthia G. Whitney, Nancy E. Messonnier, Elizabeth R. Zell, Ruth Lynfield, James L. Hadler, Lee H. Harrison et al., “Bacterial Meningitis in the United States, 1998–2007,” *New England Journal of Medicine* 364, no. 21 (2011): 2016-25.



(a)



(b)

(a) A normal human brain removed during an autopsy. (b) The brain of a patient who died from bacterial meningitis. Note the pus under the dura mater (being retracted by the forceps) and the red hemorrhagic foci on the meninges. (credit b: modification of work by the Centers for Disease Control and Prevention)

A variety of other bacteria, including *Listeria monocytogenes* and *Escherichia coli*, are also capable of causing meningitis. These bacteria cause infections of the arachnoid mater and CSF after spreading through the

circulation in blood or by spreading from an infection of the sinuses or nasopharynx. *Streptococcus agalactiae*, commonly found in the microbiota of the vagina and gastrointestinal tract, can also cause bacterial meningitis in newborns after transmission from the mother either before or during birth.

The profound inflammation caused by these microbes can result in early symptoms that include severe headache, fever, confusion, nausea, vomiting, photophobia, and stiff neck. Systemic inflammatory responses associated with some types of bacterial meningitis can lead to hemorrhaging and purpuric lesions on skin, followed by even more severe conditions that include shock, convulsions, coma, and death—in some cases, in the span of just a few hours.

Diagnosis of bacterial meningitis is best confirmed by analysis of CSF obtained by a lumbar puncture. Abnormal levels of polymorphonuclear neutrophils (PMNs) (> 10 PMNs/mm³), glucose (< 45 mg/dL), and protein (> 45 mg/dL) in the CSF are suggestive of bacterial meningitis.[\[footnote\]](#) Characteristics of specific forms of bacterial meningitis are detailed in the subsections that follow.

Popovic, T., et al. World Health Organization, “Laboratory Manual for the Diagnosis of Meningitis Caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenza*,” 1999.

Meningococcal Meningitis

Meningococcal meningitis is a serious infection caused by the gram-negative coccus *N. meningitidis*. In some cases, death can occur within a few hours of the onset of symptoms. Nonfatal cases can result in irreversible nerve damage, resulting in hearing loss and brain damage, or amputation of extremities because of tissue necrosis.

Meningococcal meningitis can infect people of any age, but its prevalence is highest among infants, adolescents, and young adults.[\[footnote\]](#) Meningococcal meningitis was once the most common cause of meningitis epidemics in human populations. This is still the case in a swath of sub-

Saharan Africa known as the meningitis belt, but meningococcal meningitis epidemics have become rare in most other regions, thanks to meningococcal vaccines. However, outbreaks can still occur in communities, schools, colleges, prisons, and other populations where people are in close direct contact.

US Centers for Disease Control and Prevention, “Meningococcal Disease,” August 5, 2015. Accessed June 28, 2015.

<http://www.cdc.gov/meningococcal/surveillance/index.html>.

N. meningitidis has a high affinity for mucosal membranes in the oropharynx and nasopharynx. Contact with respiratory secretions containing *N. meningitidis* is an effective mode of transmission. The pathogenicity of *N. meningitidis* is enhanced by virulence factors that contribute to the rapid progression of the disease. These include lipooligosaccharide (LOS) endotoxin, type IV pili for attachment to host tissues, and polysaccharide capsules that help the cells avoid phagocytosis and complement-mediated killing. Additional virulence factors include IgA protease (which breaks down IgA antibodies), the invasion factors Opa, Opc, and porin (which facilitate transcellular entry through the blood-brain barrier), iron-uptake factors (which strip heme units from hemoglobin in host cells and use them for growth), and stress proteins that protect bacteria from reactive oxygen molecules.

A unique sign of meningococcal meningitis is the formation of a petechial rash on the skin or mucous membranes, characterized by tiny, red, flat, hemorrhagic lesions. This rash, which appears soon after disease onset, is a response to LOS endotoxin and adherence virulence factors that disrupt the endothelial cells of capillaries and small veins in the skin. The blood vessel disruption triggers the formation of tiny blood clots, causing blood to leak into the surrounding tissue. As the infection progresses, the levels of virulence factors increase, and the hemorrhagic lesions can increase in size as blood continues to leak into tissues. Lesions larger than 1.0 cm usually occur in patients developing shock, as virulence factors cause increased hemorrhage and clot formation. Sepsis, as a result of systemic damage from meningococcal virulence factors, can lead to rapid multiple organ failure, shock, disseminated intravascular coagulation, and death.

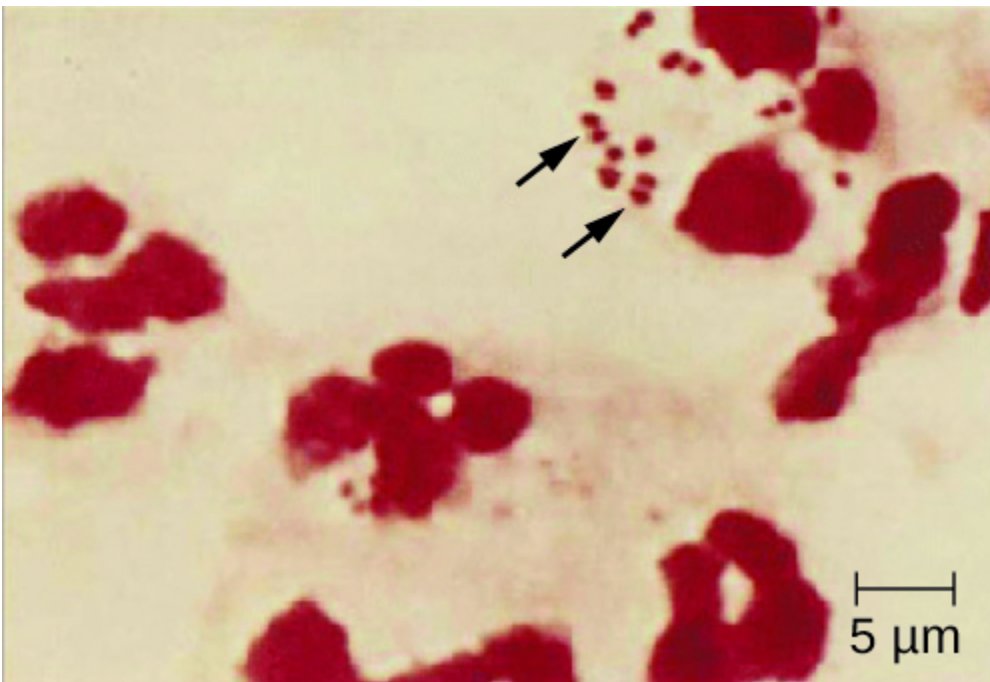
Because meningococcal meningitis progresses so rapidly, a greater variety of clinical specimens are required for the timely detection of *N. meningitidis*. Required specimens can include blood, CSF, naso- and oropharyngeal swabs, urethral and endocervical swabs, petechial aspirates, and biopsies. Safety protocols for handling and transport of specimens suspected of containing *N. meningitidis* should always be followed, since cases of fatal meningococcal disease have occurred in healthcare workers exposed to droplets or aerosols from patient specimens. Prompt presumptive diagnosis of meningococcal meningitis can occur when CSF is directly evaluated by Gram stain, revealing extra- and intracellular gram-negative diplococci with a distinctive coffee-bean microscopic morphology associated with PMNs ([link](#)). Identification can also be made directly from CSF using latex agglutination and immunochromatographic rapid diagnostic tests specific for *N. meningitidis*. Species identification can also be performed using DNA sequence-based typing schemes for hypervariable outer membrane proteins of *N. meningitidis*, which has replaced sero(sub)typing.

Meningococcal infections can be treated with antibiotic therapy, and third-generation cephalosporins are most often employed. However, because outcomes can be negative even with treatment, preventive vaccination is the best form of treatment. In 2010, countries in Africa's meningitis belt began using a new serogroup A meningococcal conjugate vaccine. This program has dramatically reduced the number of cases of meningococcal meningitis by conferring individual and herd immunity.

Twelve different capsular serotypes of *N. meningitidis* are known to exist. Serotypes A, B, C, W, X, and Y are the most prevalent worldwide. The CDC recommends that children between 11–12 years of age be vaccinated with a single dose of a quadrivalent vaccine that protects against serotypes A, C, W, and Y, with a booster at age 16.[\[footnote\]](#) An additional booster or injections of serogroup B meningococcal vaccine may be given to individuals in high-risk settings (such as epidemic outbreaks on college campuses).

US Centers for Disease Control and Prevention, "Recommended Immunization Schedule for Persons Aged 0 Through 18 Years, United

States, 2016,” February 1, 2016. Accessed on June 28, 2016.
<http://www.cdc.gov/vaccines/schedules/hcp/imz/child-adolescent.html>.



N. meningitidis (arrows) associated with neutrophils (the larger stained cells) in a gram-stained CSF sample.
(credit: modification of work by the Centers for Disease Control and Prevention)

Note:

Meningitis on Campus

College students living in dorms or communal housing are at increased risk for contracting epidemic meningitis. From 2011 to 2015, there have been at least nine meningococcal outbreaks on college campuses in the United States. These incidents involved a total of 43 students (of whom four died). [\[footnote\]](#) In spite of rapid diagnosis and aggressive antimicrobial

treatment, several of the survivors suffered from amputations or serious neurological problems.

National Meningitis Association, “Serogroup B Meningococcal Disease Outbreaks on U.S. College Campuses,” 2016. Accessed June 28, 2016. <http://www.nmaus.org/disease-prevention-information/serogroup-b-meningococcal-disease/outbreaks/>.

Prophylactic vaccination of first-year college students living in dorms is recommended by the CDC, and insurance companies now cover meningococcal vaccination for students in college dorms. Some colleges have mandated vaccination with meningococcal conjugate vaccine for certain students entering college ([link](#)).



To prevent campus outbreaks, some colleges now require students to be vaccinated against meningococcal meningitis. (credit: modification of work by James Gathany, Centers for Disease Control and Prevention)

Pneumococcal Meningitis

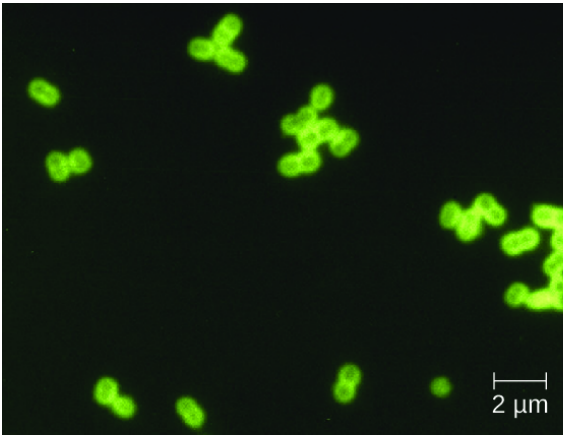
Pneumococcal meningitis is caused by the encapsulated gram-positive bacterium *S. pneumoniae* (pneumococcus, also called strep pneumo). This organism is commonly found in the microbiota of the pharynx of 30–70% of young children, depending on the sampling method, while *S. pneumoniae* can be found in fewer than 5% of healthy adults. Although it is often present without disease symptoms, this microbe can cross the blood-brain barrier in susceptible individuals. In some cases, it may also result in septicemia. Since the introduction of the Hib vaccine, *S. pneumoniae* has become the leading cause of meningitis in humans aged 2 months through adulthood.

S. pneumoniae can be identified in CSF samples using gram-stained specimens, latex agglutination, and immunochromatographic RDT specific for *S. pneumoniae*. In gram-stained samples, *S. pneumoniae* appears as gram-positive, lancet-shaped diplococci ([\[link\]](#)). Identification of *S. pneumoniae* can also be achieved using cultures of CSF and blood, and at least 93 distinct serotypes can be identified based on the quellung reaction to unique capsular polysaccharides. PCR and RT-PCR assays are also available to confirm identification.

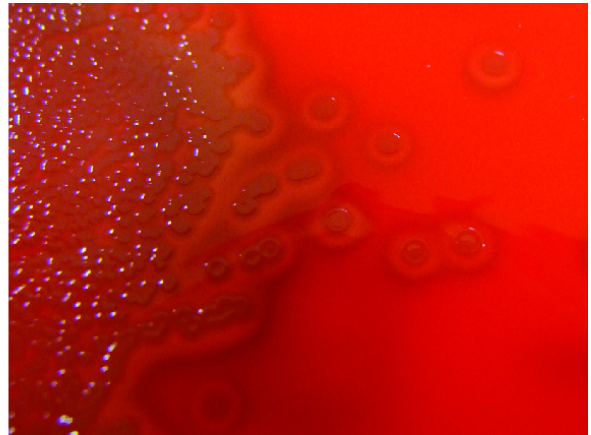
Major virulence factors produced by *S. pneumoniae* include PI-1 pilin for adherence to host cells (pneumococcal adherence) and virulence factor B (PavB) for attachment to cells of the respiratory tract; choline-binding proteins (cbpA) that bind to epithelial cells and interfere with immune factors IgA and C3; and the cytoplasmic bacterial toxin pneumolysin that triggers an inflammatory response.

With the emergence of drug-resistant strains of *S. pneumoniae*, pneumococcal meningitis is typically treated with broad-spectrum antibiotics, such as levofloxacin, cefotaxime, penicillin, or other β -lactam

antibiotics. The two available pneumococcal vaccines are described in [Bacterial Infections of the Respiratory Tract](#).



(a)



(b)

(a) Digitally colorized fluorescent antibody stained micrograph of *Streptococcus pneumoniae* in CSF. (b) *S. pneumoniae* growing on blood agar. (credit a: modification of work by the Centers for Disease Control and Prevention; credit b: modification of work by Nathan Reading)

***Haemophilus influenzae* Type b**

Meningitis due to *H. influenzae* serotype b (Hib), an encapsulated pleomorphic gram-negative coccobacilli, is now uncommon in most countries, because of the use of the effective Hib vaccine. Without the use of the Hib vaccine, *H. influenzae* can be the primary cause of meningitis in children 2 months thru 5 years of age. *H. influenzae* can be found in the throats of healthy individuals, including infants and young children. By five years of age, most children have developed immunity to this microbe. Infants older than 2 months of age, however, do not produce a sufficient protective antibody response and are susceptible to serious disease. The

intracranial pressure caused by this infection leads to a 5% mortality rate and 20% incidence of deafness or brain damage in survivors.[\[footnote\]](#)

United States Department of Health and Human Services, “Hib (Haemophilus Influenzae Type B),” Accessed June 28, 2016.

<http://www.vaccines.gov/diseases/hib/#>.

H. influenzae produces at least 16 different virulence factors, including LOS, which triggers inflammation, and *Haemophilus* adhesion and penetration factor (Hap), which aids in attachment and invasion into respiratory epithelial cells. The bacterium also has a polysaccharide capsule that helps it avoid phagocytosis, as well as factors such as IgA1 protease and P2 protein that allow it to evade antibodies secreted from mucous membranes. In addition, factors such as hemoglobin-binding protein (Hgp) and transferrin-binding protein (Tbp) acquire iron from hemoglobin and transferrin, respectively, for bacterial growth.

Preliminary diagnosis of *H. influenzae* infections can be made by direct PCR and a smear of CSF. Stained smears will reveal intracellular and extracellular PMNs with small, pleomorphic, gram-negative coccobacilli or filamentous forms that are characteristic of *H. influenzae*. Initial confirmation of this genus can be based on its fastidious growth on chocolate agar. Identification is confirmed with requirements for exogenous biochemical growth cofactors NAD and heme (by MALDI-TOF), latex agglutination, and RT-PCR.

Meningitis caused by *H. influenzae* is usually treated with doxycycline, fluoroquinolones, second- and third-generation cephalosporins, and carbapenems. The best means of preventing *H. influenza* infection is with the use of the Hib polysaccharide conjugate vaccine. It is recommended that all children receive this vaccine at 2, 4, and 6 months of age, with a final booster dose at 12 to 15 months of age.[\[footnote\]](#)

US Centers for Disease Control and Prevention, “Meningococcal Disease, Disease Trends,” 2015. Accessed September 13, 2016.

<http://www.cdc.gov/meningococcal/surveillance/index.html>.

Neonatal Meningitis

S. agalactiae, Group B streptococcus (GBS), is an encapsulated gram-positive bacterium that is the most common cause of **neonatal meningitis**, a term that refers to meningitis occurring in babies up to 3 months of age.

[\[footnote\]](#) *S. agalactiae* can also cause meningitis in people of all ages and can be found in the urogenital and gastrointestinal microbiota of about 10–30% of humans.

Thigpen, Michael C., Cynthia G. Whitney, Nancy E. Messonnier, Elizabeth R. Zell, Ruth Lynfield, James L. Hadler, Lee H. Harrison et al., “Bacterial Meningitis in the United States, 1998–2007,” *New England Journal of Medicine* 364, no. 21 (2011): 2016-25.

Neonatal infection occurs as either early onset or late-onset disease. Early onset disease is defined as occurring in infants up to 7 days old. The infant initially becomes infected by *S. agalactiae* during childbirth, when the bacteria may be transferred from the mother’s vagina. Incidence of early onset neonatal meningitis can be greatly reduced by giving intravenous antibiotics to the mother during labor.

Late-onset neonatal meningitis occurs in infants between 1 week and 3 months of age. Infants born to mothers with *S. agalactiae* in the urogenital tract have a higher risk of late-onset meningitis, but late-onset infections can be transmitted from sources other than the mother; often, the source of infection is unknown. Infants who are born prematurely (before 37 weeks of pregnancy) or to mothers who develop a fever also have a greater risk of contracting late-onset neonatal meningitis.

Signs and symptoms of early onset disease include temperature instability, apnea (cessation of breathing), bradycardia (slow heart rate), hypotension, difficulty feeding, irritability, and limpness. When asleep, the baby may be difficult to wake up. Symptoms of late-onset disease are more likely to include seizures, bulging fontanel (soft spot), stiff neck, hemiparesis (weakness on one side of the body), and **opisthotonos** (rigid body with arched back and head thrown backward).

S. agalactiae produces at least 12 virulence factors that include FbsA that attaches to host cell surface proteins, PI-1 pili that promotes the invasion of human endothelial cells, a polysaccharide capsule that prevents the activation of the alternative complement pathway and inhibits phagocytosis,

and the toxin CAMP factor, which forms pores in host cell membranes and binds to IgG and IgM antibodies.

Diagnosis of neonatal meningitis is often, but not uniformly, confirmed by positive results from cultures of CSF or blood. Tests include routine culture, antigen detection by enzyme immunoassay, serotyping of different capsule types, PCR, and RT-PCR. It is typically treated with β -lactam antibiotics such as intravenous penicillin or ampicillin plus gentamicin. Even with treatment, roughly 10% mortality is seen in infected neonates.[\[footnote\]](#)

Thigpen, Michael C., Cynthia G. Whitney, Nancy E. Messonnier, Elizabeth R. Zell, Ruth Lynfield, James L. Hadler, Lee H. Harrison et al., “Bacterial Meningitis in the United States, 1998–2007,” *New England Journal of Medicine* 364, no. 21 (2011): 2016-25; Heath, Paul T., Gail Balfour, Abbie M. Weisner, Androulla Efstratiou, Theresa L. Lamagni, Helen Tighe, Liam AF O’Connell et al., “Group B Streptococcal Disease in UK and Irish Infants Younger than 90 Days,” *The Lancet* 363, no. 9405 (2004): 292-4.

Note:

- Which groups are most vulnerable to each of the bacterial meningitis diseases?
- For which of the bacterial meningitis diseases are there vaccines presently available?
- Which organism can cause epidemic meningitis?

***Clostridium*-Associated Diseases**

Species in the genus *Clostridium* are gram-positive, endospore-forming rods that are obligate anaerobes. Endospores of *Clostridium* spp. are widespread in nature, commonly found in soil, water, feces, sewage, and marine sediments. *Clostridium* spp. produce more types of protein exotoxins than any other bacterial genus, including two exotoxins with protease activity that are the most potent known biological toxins:

botulinum neurotoxin (BoNT) and tetanus neurotoxin (TeNT). These two toxins have lethal doses of 0.2–10 ng per kg body weight.

BoNT can be produced by unique strains of *C. butyricum*, and *C. baratii*; however, it is primarily associated with *C. botulinum* and the condition of botulism. TeNT, which causes tetanus, is only produced by *C. tetani*. These powerful neural exotoxins are the primary virulence factors for these pathogens. The mode of action for these toxins was described in [Virulence Factors of Bacterial and Viral Pathogens](#) and illustrated in [\[link\]](#).

Diagnosis of tetanus or botulism typically involves bioassays that detect the presence of BoNT and TeNT in fecal specimens, blood (serum), or suspect foods. In addition, both *C. botulinum* and *C. tetani* can be isolated and cultured using commercially available media for anaerobes. ELISA and RT-PCR tests are also available.

Tetanus

Tetanus is a noncommunicable disease characterized by uncontrollable muscle spasms (contractions) caused by the action of TeNT. It generally occurs when *C. tetani* infects a wound and produces TeNT, which rapidly binds to neural tissue, resulting in an intoxication (poisoning) of neurons. Depending on the site and extent of infection, cases of tetanus can be described as localized, cephalic, or generalized. Generalized tetanus that occurs in a newborn is called neonatal tetanus.

Localized tetanus occurs when TeNT only affects the muscle groups close to the injury site. There is no CNS involvement, and the symptoms are usually mild, with localized muscle spasms caused by a dysfunction in the surrounding neurons. Individuals with partial immunity—especially previously vaccinated individuals who neglect to get the recommended booster shots—are most likely to develop localized tetanus as a result of *C. tetani* infecting a puncture wound.

Cephalic tetanus is a rare, localized form of tetanus generally associated with wounds on the head or face. In rare cases, it has occurred in cases of

otitis media (middle ear infection). Cephalic tetanus often results in patients seeing double images, because of the spasms affecting the muscles that control eye movement.

Both localized and cephalic tetanus may progress to generalized tetanus—a much more serious condition—if TeNT is able to spread further into body tissues. In generalized tetanus, TeNT enters neurons of the PNS. From there, TeNT travels from the site of the wound, usually on an extremity of the body, retrograde (back up) to inhibitory neurons in the CNS. There, it prevents the release of gamma aminobutyric acid (GABA), the neurotransmitter responsible for muscle relaxation. The resulting muscle spasms often first occur in the jaw muscles, leading to the characteristic symptom of lockjaw (inability to open the mouth). As the toxin progressively continues to block neurotransmitter release, other muscles become involved, resulting in uncontrollable, sudden muscle spasms that are powerful enough to cause tendons to rupture and bones to fracture. Spasms in the muscles in the neck, back, and legs may cause the body to form a rigid, stiff arch, a posture called opisthotonos ([\[link\]](#)). Spasms in the larynx, diaphragm, and muscles of the chest restrict the patient's ability to swallow and breathe, eventually leading to death by asphyxiation (insufficient supply of oxygen).

Neonatal tetanus typically occurs when the stump of the umbilical cord is contaminated with spores of *C. tetani* after delivery. Although this condition is rare in the United States, neonatal tetanus is a major cause of infant mortality in countries that lack maternal immunization for tetanus and where birth often occurs in unsanitary conditions. At the end of the first week of life, infected infants become irritable, feed poorly, and develop rigidity with spasms. Neonatal tetanus has a very poor prognosis with a mortality rate of 70%–100%.[\[footnote\]](#)

UNFPA, UNICEF WHO, “Maternal and Neonatal Tetanus Elimination by 2005,” 2000.

http://www.unicef.org/immunization/files/MNTE_strategy_paper.pdf.

Treatment for patients with tetanus includes assisted breathing through the use of a ventilator, wound debridement, fluid balance, and antibiotic therapy with metronidazole or penicillin to halt the growth of *C. tetani*. In addition,

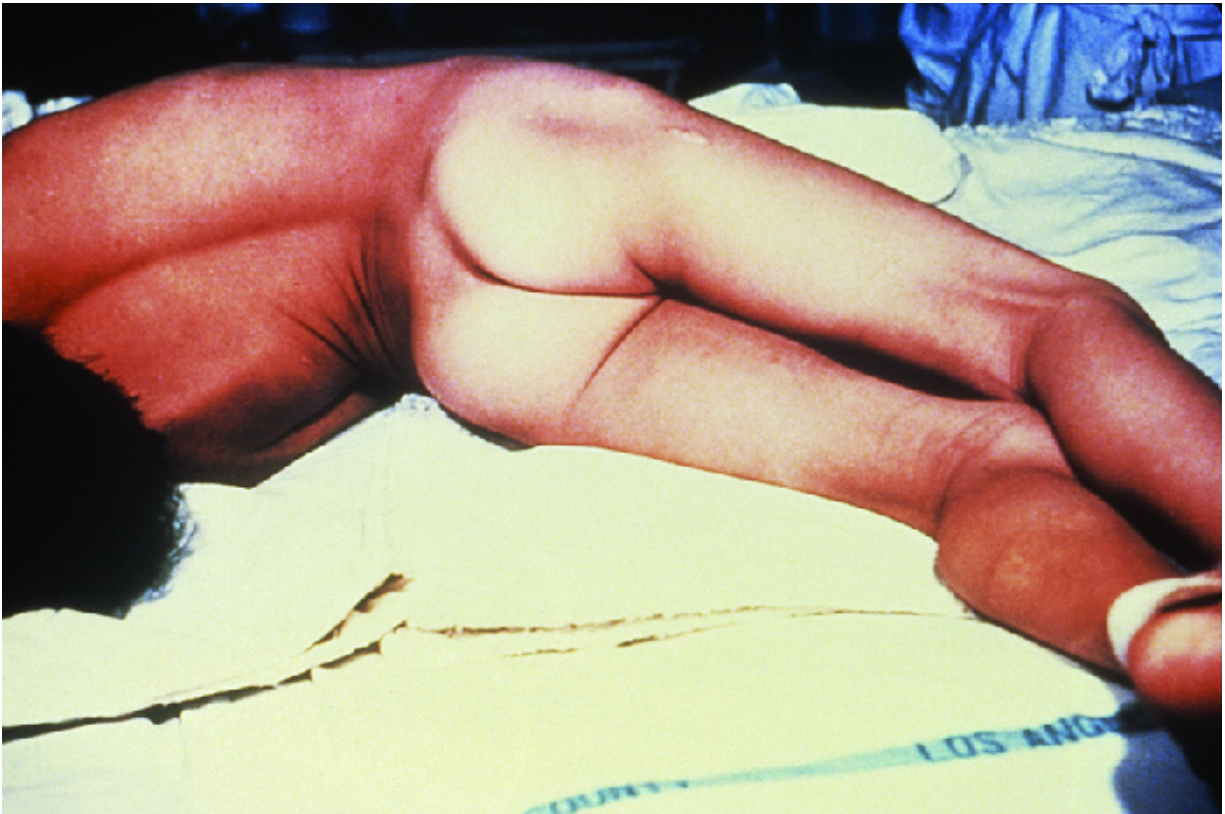
patients are treated with TeNT antitoxin, preferably in the form of human immunoglobulin to neutralize nonfixed toxin and benzodiazepines to enhance the effect of GABA for muscle relaxation and anxiety.

A tetanus toxoid (TT) vaccine is available for protection and prevention of tetanus. It is the T component of vaccines such as DTaP, Tdap, and Td. The CDC recommends children receive doses of the DTaP vaccine at 2, 4, 6, and 15–18 months of age and another at 4–6 years of age. One dose of Td is recommended for adolescents and adults as a TT booster every 10 years.

[\[footnote\]](#)

US Centers for Disease Control and Prevention, “Tetanus Vaccination,” 2013. Accessed June 29, 2016.

<http://www.cdc.gov/tetanus/vaccination.html>.



A tetanus patient exhibiting the rigid body posture known as opisthotonos. (credit: Centers for Disease Control and Prevention)

Botulism

Botulism is a rare but frequently fatal illness caused by intoxication by BoNT. It can occur either as the result of an infection by *C. botulinum*, in which case the bacteria produce BoNT *in vivo*, or as the result of a direct introduction of BoNT into tissues.

Infection and production of BoNT *in vivo* can result in wound botulism, infant botulism, and adult intestinal toxemia. Wound botulism typically occurs when *C. botulinum* is introduced directly into a wound after a traumatic injury, deep puncture wound, or injection site. Infant botulism, which occurs in infants younger than 1 year of age, and adult intestinal toxemia, which occurs in immunocompromised adults, results from ingesting *C. botulinum* endospores in food. The endospores germinate in the body, resulting in the production of BoNT in the intestinal tract.

Intoxications occur when BoNT is produced outside the body and then introduced directly into the body through food (foodborne botulism), air (inhalation botulism), or a clinical procedure (iatrogenic botulism). Foodborne botulism, the most common of these forms, occurs when BoNT is produced in contaminated food and then ingested along with the food (recall [Case in Point: A Streak of Bad Potluck](#)). Inhalation botulism is rare because BoNT is unstable as an aerosol and does not occur in nature; however, it can be produced in the laboratory and was used (unsuccessfully) as a bioweapon by terrorists in Japan in the 1990s. A few cases of accidental inhalation botulism have also occurred. Iatrogenic botulism is also rare; it is associated with injections of BoNT used for cosmetic purposes (see [Micro Connections: Medicinal Uses of Botulinum Toxin](#)).

When BoNT enters the bloodstream in the gastrointestinal tract, wound, or lungs, it is transferred to the neuromuscular junctions of motor neurons where it binds irreversibly to presynaptic membranes and prevents the release of acetylcholine from the presynaptic terminal of motor neurons into the neuromuscular junction. The consequence of preventing acetylcholine

release is the loss of muscle activity, leading to muscle relaxation and eventually paralysis.

If BoNT is absorbed through the gastrointestinal tract, early symptoms of botulism include blurred vision, drooping eyelids, difficulty swallowing, abdominal cramps, nausea, vomiting, constipation, or possibly diarrhea. This is followed by progressive flaccid paralysis, a gradual weakening and loss of control over the muscles. A patient's experience can be particularly terrifying, because hearing remains normal, consciousness is not lost, and he or she is fully aware of the progression of his or her condition. In infants, notable signs of botulism include weak cry, decreased ability to suckle, and hypotonia (limpness of head or body). Eventually, botulism ends in death from respiratory failure caused by the progressive paralysis of the muscles of the upper airway, diaphragm, and chest.

Botulism is treated with an antitoxin specific for BoNT. If administered in time, the antitoxin stops the progression of paralysis but does not reverse it. Once the antitoxin has been administered, the patient will slowly regain neurological function, but this may take several weeks or months, depending on the severity of the case. During recovery, patients generally must remain hospitalized and receive breathing assistance through a ventilator.

Note:

- How frequently should the tetanus vaccination be updated in adults?
- What are the most common causes of botulism?
- Why is botulism not treated with an antibiotic?

Note:

Medicinal Uses of Botulinum Toxin

Although it is the most toxic biological material known to man, botulinum toxin is often intentionally injected into people to treat other conditions.

Type A botulinum toxin is used cosmetically to reduce wrinkles. The injection of minute quantities of this toxin into the face causes the relaxation of facial muscles, thereby giving the skin a smoother appearance. Eyelid twitching and crossed eyes can also be treated with botulinum toxin injections. Other uses of this toxin include the treatment of hyperhidrosis (excessive sweating). In fact, botulinum toxin can be used to moderate the effects of several other apparently nonmicrobial diseases involving inappropriate nerve function. Such diseases include cerebral palsy, multiple sclerosis, and Parkinson's disease. Each of these diseases is characterized by a loss of control over muscle contractions; treatment with botulinum toxin serves to relax contracted muscles.

Listeriosis

Listeria monocytogenes is a nonencapsulated, nonsporulating, gram-positive rod and a foodborne pathogen that causes **listeriosis**. At-risk groups include pregnant women, neonates, the elderly, and the immunocompromised (recall the Clinical Focus case studies in [Microbial Growth](#) and [Microbial Mechanisms of Pathogenicity](#)). Listeriosis leads to meningitis in about 20% of cases, particularly neonates and patients over the age of 60. The CDC identifies listeriosis as the third leading cause of death due to foodborne illness, with overall mortality rates reaching 16%. [\[footnote\]](#) In pregnant women, listeriosis can also cause spontaneous abortion in pregnant women because of the pathogen's unique ability to cross the placenta.

Scallan, Elaine, Robert M. Hoekstra, Frederick J. Angulo, Robert V. Tauxe, Marc-Alain Widdowson, Sharon L. Roy, Jeffery L. Jones, and Patricia M. Griffin, "Foodborne Illness Acquired in the United States—Major Pathogens," *Emerging Infectious Diseases* 17, no. 1 (2011): 7-15.

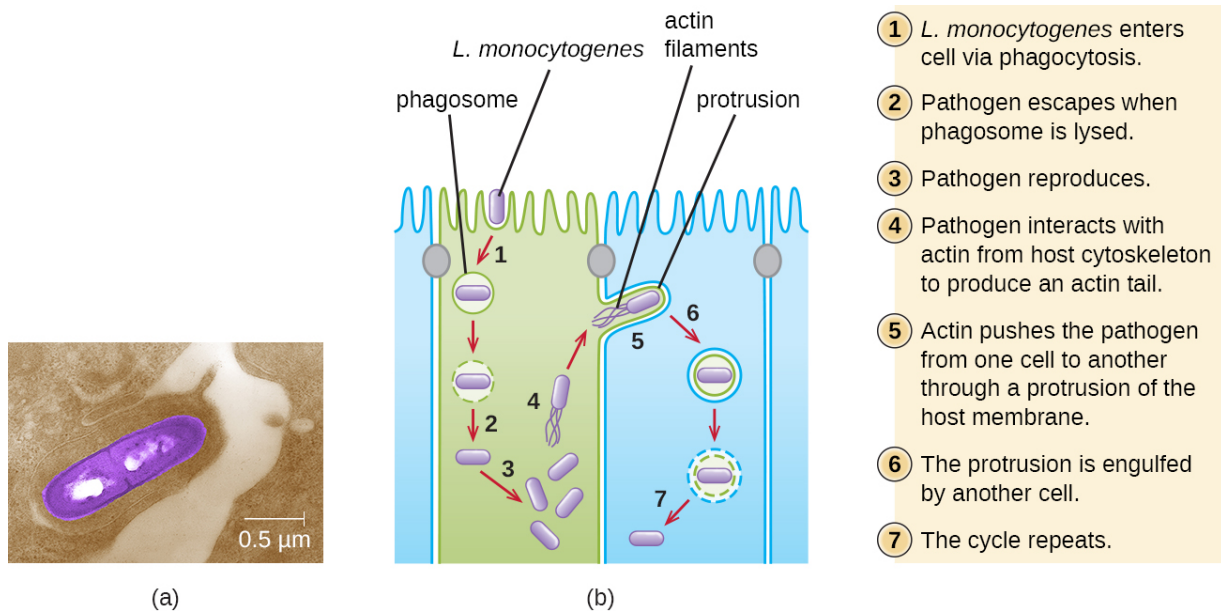
L. monocytogenes is generally introduced into food items by contamination with soil or animal manure used as fertilizer. Foods commonly associated with listeriosis include fresh fruits and vegetables, frozen vegetables, processed meats, soft cheeses, and raw milk. [\[footnote\]](#) Unlike most other foodborne pathogens, *Listeria* is able to grow at temperatures between 0 °C and 50 °C, and can therefore continue to grow, even in refrigerated foods.

US Centers for Disease Control and Prevention, “*Listeria* Outbreaks,” 2016. Accessed June 29, 2016.
<https://www.cdc.gov/listeria/outbreaks/index.html>.

Ingestion of contaminated food leads initially to infection of the gastrointestinal tract. However, *L. monocytogenes* produces several unique virulence factors that allow it to cross the intestinal barrier and spread to other body systems. Surface proteins called internalins (InlA and InlB) help *L. monocytogenes* invade nonphagocytic cells and tissues, penetrating the intestinal wall and becoming disseminating through the circulatory and lymphatic systems. Internalins also enable *L. monocytogenes* to breach other important barriers, including the blood-brain barrier and the placenta. Within tissues, *L. monocytogenes* uses other proteins called listeriolysin O and ActA to facilitate intercellular movement, allowing the infection to spread from cell to cell ([link](#)).

L. monocytogenes is usually identified by cultivation of samples from a normally sterile site (e.g., blood or CSF). Recovery of viable organisms can be enhanced using cold enrichment by incubating samples in a broth at 4 °C for a week or more. Distinguishing types and subtypes of *L. monocytogenes*—an important step for diagnosis and epidemiology—is typically done using pulsed-field gel electrophoresis. Identification can also be achieved using chemiluminescence DNA probe assays and MALDI-TOF.

Treatment for listeriosis involves antibiotic therapy, most commonly with ampicillin and gentamicin. There is no vaccine available.



(a) An electron micrograph of *Listeria monocytogenes* infecting a host cell. (b) *Listeria* is able to use host cell components to cause infection. For example, phagocytosis allows it to enter host cells, and the host's cytoskeleton provides the materials to help the pathogen move to other cells. (credit a: modification of work by the Centers for Disease Control and Prevention; credit b: modification of work by Keith Ireton)

Note:

- How does *Listeria* enter the nervous system?

Hansen's Disease (Leprosy)

Hansen's disease (also known as **leprosy**) is caused by a long, thin, filamentous rod-shaped bacterium *Mycobacterium leprae*, an obligate

intracellular pathogen. *M. leprae* is classified as gram-positive bacteria, but it is best visualized microscopically with an acid-fast stain and is generally referred to as an acid-fast bacterium. Hansen's disease affects the PNS, leading to permanent damage and loss of appendages or other body parts.

Hansen's disease is communicable but not highly contagious; approximately 95% of the human population cannot be easily infected because they have a natural immunity to *M. leprae*. Person-to-person transmission occurs by inhalation into nasal mucosa or prolonged and repeated contact with infected skin. Armadillos, one of only five mammals susceptible to Hansen's disease, have also been implicated in transmission of some cases.[\[footnote\]](#)

Sharma, Rahul, Pushpendra Singh, W. J. Loughry, J. Mitchell Lockhart, W. Barry Inman, Malcolm S. Duthie, Maria T. Pena et al., "Zoonotic Leprosy in the Southeastern United States," *Emerging Infectious Diseases* 21, no. 12 (2015): 2127-34.

In the human body, *M. leprae* grows best at the cooler temperatures found in peripheral tissues like the nose, toes, fingers, and ears. Some of the virulence factors that contribute to *M. leprae*'s pathogenicity are located on the capsule and cell wall of the bacterium. These virulence factors enable it to bind to and invade Schwann cells, resulting in progressive demyelination that gradually destroys neurons of the PNS. The loss of neuronal function leads to hypoesthesia (numbness) in infected lesions. *M. leprae* is readily phagocytized by macrophages but is able to survive within macrophages in part by neutralizing reactive oxygen species produced in the oxidative burst of the phagolysosome. Like *L. monocytogenes*, *M. leprae* also can move directly between macrophages to avoid clearance by immune factors.

The extent of the disease is related to the immune response of the patient. Initial symptoms may not appear for as long as 2 to 5 years after infection. These often begin with small, blanched, numb areas of the skin. In most individuals, these will resolve spontaneously, but some cases may progress to a more serious form of the disease. Tuberculoid (paucibacillary) Hansen's disease is marked by the presence of relatively few (three or less) flat, blanched skin lesions with small nodules at the edges and few bacteria present in the lesion. Although these lesions can persist for years or

decades, the bacteria are held in check by an effective immune response including cell-mediated cytotoxicity. Individuals who are unable to contain the infection may later develop lepromatous (multibacillary) Hansen's disease. This is a progressive form of the disease characterized by nodules filled with acid-fast bacilli and macrophages. Impaired function of infected Schwann cells leads to peripheral nerve damage, resulting in sensory loss that leads to ulcers, deformities, and fractures. Damage to the ulnar nerve (in the wrist) by *M. leprae* is one of the most common causes of crippling of the hand. In some cases, chronic tissue damage can ultimately lead to loss of fingers or toes. When mucosal tissues are also involved, disfiguring lesions of the nose and face can also occur ([\[link\]](#)).

Hansen's disease is diagnosed on the basis of clinical signs and symptoms of the disease, and confirmed by the presence of acid-fast bacilli on skin smears or in skin biopsy specimens ([\[link\]](#)). *M. leprae* does not grow *in vitro* on any known laboratory media, but it can be identified by culturing *in vivo* in the footpads of laboratory mice or armadillos. Where needed, PCR and genotyping of *M. leprae* DNA in infected human tissue may be performed for diagnosis and epidemiology.

Hansen's disease responds well to treatment and, if diagnosed and treated early, does not cause disability. In the United States, most patients with Hansen's disease are treated in ambulatory care clinics in major cities by the National Hansen's Disease program, the only institution in the United States exclusively devoted to Hansen's disease. Since 1995, WHO has made multidrug therapy for Hansen's disease available free of charge to all patients worldwide. As a result, global prevalence of Hansen's disease has declined from about 5.2 million cases in 1985 to roughly 176,000 in 2014. [\[footnote\]](#) Multidrug therapy consists of dapsone and rifampicin for all patients and a third drug, clofazimine, for patients with multibacillary disease.

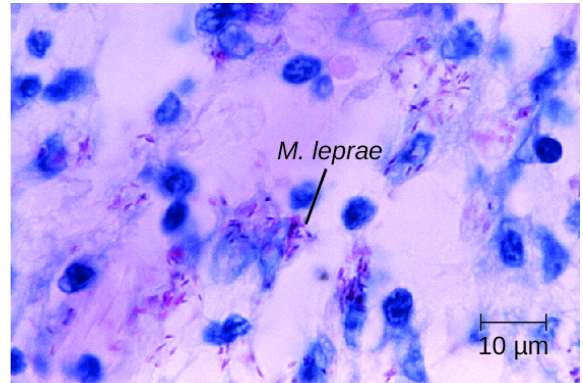
World Health Organization, "Leprosy Fact Sheet," 2016. Accessed September 13, 2016. <http://www.who.int/mediacentre/factsheets/fs101/en/>.

Currently, there is no universally accepted vaccine for Hansen's disease. India and Brazil use a tuberculosis vaccine against Hansen's disease because both diseases are caused by species of *Mycobacterium*. The

effectiveness of this method is questionable, however, since it appears that the vaccine works in some populations but not in others.



(a)



(b)

(a) The nose of a patient with Hansen's disease. Note the lepromatous/multibacillary lesions around the nostril. (b) Hansen's disease is caused by *Mycobacterium leprae*, a gram-positive bacillus. (credit a, b: modifications of work by the Centers for Disease Control and Prevention)

Note:

- What prevents the progression from tuberculoid to lepromatous leprosy?
- Why does Hansen's disease typically affect the nerves of the extremities?

Note:

Leper Colonies

Disfiguring, deadly diseases like leprosy have historically been stigmatized in many cultures. Before leprosy was understood, victims were often isolated in leper colonies, a practice mentioned frequently in ancient texts, including the Bible. But leper colonies are not just an artifact of the ancient world. In Hawaii, a leper colony established in the late nineteenth century persisted until the mid-twentieth century, its residents forced to live in deplorable conditions.^[footnote] Although leprosy is a communicable disease, it is not considered contagious (easily communicable), and it certainly does not pose enough of a threat to justify the permanent isolation of its victims. Today, we reserve the practices of isolation and quarantine to patients with more dangerous diseases, such as Ebola or multiple-drug-resistant bacteria like *Mycobacterium tuberculosis* and *Staphylococcus aureus*. The ethical argument for this practice is that isolating infected patients is necessary to prevent the transmission and spread of highly contagious diseases—even when it goes against the wishes of the patient. National Park Service, “A Brief History of Kalaupapa,” Accessed February 2, 2016. <http://www.nps.gov/kala/learn/historyculture/a-brief-history-of-kalaupapa.htm>.

Of course, it is much easier to justify the practice of temporary, clinical quarantining than permanent social segregation, as occurred in leper colonies. In the 1980s, there were calls by some groups to establish camps for people infected with AIDS. Although this idea was never actually implemented, it begs the question—where do we draw the line? Are permanent isolation camps or colonies ever medically or socially justifiable? Suppose there were an outbreak of a fatal, contagious disease for which there is no treatment. Would it be justifiable to impose social isolation on those afflicted with the disease? How would we balance the rights of the infected with the risk they pose to others? To what extent should society expect individuals to put their own health at risk for the sake of treating others humanely?

Note:**Bacterial Infections of the Nervous System**

Despite the formidable defenses protecting the nervous system, a number of bacterial pathogens are known to cause serious infections of the CNS or

PNS. Unfortunately, these infections are often serious and life threatening. [\[link\]](#) summarizes some important infections of the nervous system.

Bacterial Infections of the Nervous System					
Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs	Vaccine
Botulism	<i>Clostridium botulinum</i>	Blurred vision, drooping eyelids, difficulty swallowing and breathing, nausea, vomiting, often fatal	Ingestion of preformed toxin in food, ingestion of endospores in food by infants or immunocompromised adults, bacterium introduced via wound or injection	Antitoxin; penicillin (for wound botulism)	None
Hansen's disease (leprosy)	<i>Mycobacterium leprae</i>	Hypopigmented skin, skin lesions, and nodules, loss of peripheral nerve function, loss of fingers, toes, and extremities	Inhalation, possible transmissible from armadillos to humans	Dapsone, rifampin, clofazimine	None
<i>Haemophilus influenzae</i> type b meningitis	<i>Haemophilus influenzae</i>	Nausea, vomiting, photophobia, stiff neck, confusion	Direct contact, inhalation of aerosols	Doxycycline, fluoroquinolones, second- and third-generation cephalosporins, and carbapenems	Hib vaccine
Listeriosis	<i>Listeria monocytogenes</i>	Initial flu-like symptoms, sepsis and potentially fatal meningitis in susceptible individuals, miscarriage in pregnant women	Bacterium ingested with contaminated food or water	Ampicillin, gentamicin	None
Meningococcal meningitis	<i>Neisseria meningitidis</i>	Nausea, vomiting, photophobia, stiff neck, confusion; often fatal	Direct contact	Cephalosporins or penicillins	Meningococcal conjugate
Neonatal meningitis	<i>Streptococcus agalactiae</i>	Temperature instability, apnea, bradycardia, hypotension, feeding difficulty, irritability, limpness, seizures, bulging fontanel, stiff neck, opisthotonos, hemiparesis, often fatal	Direct contact in birth canal	Ampicillin plus gentamicin, cefotaxime, or both	None
Pneumococcal meningitis	<i>Streptococcus pneumoniae</i>	Nausea, vomiting, photophobia, stiff neck, confusion, often fatal	Direct contact, aerosols	Cephalosporins, penicillin	Pneumococcal vaccines
Tetanus	<i>Clostridium tetani</i>	Progressive spasmodic paralysis starting with the jaw, often fatal	Bacterium introduced in puncture wound	Penicillin, antitoxin	DTaP, Tdap

Key Concepts and Summary

- **Bacterial meningitis** can be caused by several species of encapsulated bacteria, including *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Streptococcus agalactiae* (group B streptococci). *H. influenzae* affects primarily young children and neonates, *N. meningitidis* is the only communicable pathogen and mostly affects children and young adults, *S. pneumoniae* affects mostly young children, and *S. agalactiae* affects newborns during or shortly after birth.
- Symptoms of bacterial meningitis include fever, neck stiffness, headache, confusion, convulsions, coma, and death.
- Diagnosis of bacterial meningitis is made through observations and culture of organisms in CSF. Bacterial meningitis is treated with antibiotics. *H. influenzae* and *N. meningitidis* have vaccines available.
- *Clostridium* species cause neurological diseases, including **botulism** and **tetanus**, by producing potent neurotoxins that interfere with neurotransmitter release. The PNS is typically affected. Treatment of *Clostridium* infection is effective only through early diagnosis with administration of antibiotics to control the infection and antitoxins to neutralize the endotoxin before they enter cells.
- *Listeria monocytogenes* is a foodborne pathogen that can infect the CNS, causing meningitis. The infection can be spread through the placenta to a fetus. Diagnosis is through culture of blood or CSF. Treatment is with antibiotics and there is no vaccine.
- **Hansen's disease (leprosy)** is caused by the intracellular parasite *Mycobacterium leprae*. Infections cause demyelination of neurons, resulting in decreased sensation in peripheral appendages and body sites. Treatment is with multi-drug antibiotic therapy, and there is no universally recognized vaccine.

Multiple Choice

Exercise:

Problem:

Which of the following organisms causes epidemic meningitis cases at college campuses?

- a. *Haemophilus influenzae* type b
- b. *Neisseria meningitidis*
- c. *Streptococcus pneumoniae*
- d. *Listeria monocytogenes*

Solution:

B

Exercise:**Problem:**

Which of the following is the most common cause of neonatal meningitis?

- a. *Haemophilus influenzae b*
- b. *Streptococcus agalactiae*
- c. *Neisseria meningitidis*
- d. *Streptococcus pneumoniae*

Solution:

B

Exercise:**Problem:**

What sign/symptom would NOT be associated with infant botulism?

- a. difficulty suckling
- b. limp body

- c. stiff neck
- d. weak cry

Solution:

C

Exercise:

Problem:

Which of the following can NOT be prevented with a vaccine?

- a. tetanus
- b. pneumococcal meningitis
- c. meningococcal meningitis
- d. listeriosis

Solution:

D

Exercise:

Problem: How is leprosy primarily transmitted from person to person?

- a. contaminated toilet seats
- b. shaking hands
- c. blowing nose
- d. sexual intercourse

Solution:

C

Fill in the Blank

Exercise:

Problem:

The form of meningitis that can cause epidemics is caused by the pathogen _____.

Solution:

Neisseria meningitidis

Exercise:

Problem:

The symptoms of tetanus are caused by the neurotoxin _____.

Solution:

tetanospasmin

Exercise:

Problem: _____ is another name for leprosy.

Solution:

Hansen's disease

Exercise:

Problem:

Botulism prevents the release of the neurotransmitter _____.

Solution:

acetylcholine

Exercise:

Problem:

_____ is a neurological disease that can be prevented with the DTaP vaccine.

Solution:

Tetanus

Exercise:**Problem:**

Tetanus patients exhibit _____ when muscle spasms causes them to arch their backs.

Solution:

opisthotonos

Short Answer**Exercise:****Problem:**

A physician suspects the lesion and pustule pictured here are indicative of tuberculoid leprosy. If the diagnosis is correct, what microorganism would be found in a skin biopsy?



(credit: Centers for Disease Control and Prevention)

Critical Thinking

Exercise:

Problem: Explain how tetanospasmin functions to cause disease.

Exercise:

Problem:

The most common causes of bacterial meningitis can be the result of infection by three very different bacteria. Which bacteria are they and how are these microbes similar to each other?

Exercise:

Problem:

Explain how infant botulism is different than foodborne botulism.

Acellular Diseases of the Nervous System

LEARNING OBJECTIVES

- Identify the most common acellular pathogens that can cause infections of the nervous system
- Compare the major characteristics of specific viral diseases affecting the nervous system

A number of different viruses and subviral particles can cause diseases that affect the nervous system. Viral diseases tend to be more common than bacterial infections of the nervous system today. Fortunately, viral infections are generally milder than their bacterial counterparts and often spontaneously resolve. Some of the more important acellular pathogens of the nervous system are described in this section.

Viral Meningitis

Although it is much more common than bacterial meningitis, viral meningitis is typically less severe. Many different viruses can lead to meningitis as a sequela of the primary infection, including those that cause herpes, influenza, measles, and mumps. Most cases of viral meningitis spontaneously resolve, but severe cases do occur.

Arboviral Encephalitis

Several types of insect-borne viruses can cause encephalitis. Collectively, these viruses are referred to as arboviruses (because they are arthropod-borne), and the diseases they cause are described as **arboviral encephalitis**. Most arboviruses are endemic to specific geographical regions. Arboviral encephalitis diseases found in the United States include eastern equine encephalitis (EEE), western equine encephalitis (WEE), St. Louis encephalitis, and West Nile encephalitis (WNE). Expansion of arboviruses beyond their endemic regions sometimes occurs, generally as a result of environmental changes that are favorable to the virus or its vector. Increased travel of infected humans, animals, or vectors has also allowed arboviruses to spread into new regions.

In most cases, arboviral infections are asymptomatic or lead to a mild disease. However, when symptoms do occur, they include high fever, chills, headaches, vomiting, diarrhea, and restlessness. In elderly patients, severe arboviral encephalitis can rapidly lead to convulsions, coma, and death.

Mosquitoes are the most common biological vectors for arboviruses, which tend to be enveloped ssRNA viruses. Thus, prevention of arboviral infections is best achieved by avoiding mosquitoes—using insect repellent, wearing long pants and sleeves, sleeping in well-screened rooms, using bed nets, etc.

Diagnosis of arboviral encephalitis is based on clinical symptoms and serologic testing of serum or CSF. There are no antiviral drugs to treat any of these arboviral diseases, so treatment consists of supportive care and management of symptoms.

Eastern equine encephalitis (EEE) is caused by eastern equine encephalitis virus (EEEV), which can cause severe disease in horses and humans. Birds are reservoirs for EEEV with accidental transmission to horses and humans by *Aedes*, *Coquilleltidia*, and *Culex* species of mosquitoes. Neither horses nor humans serve as reservoirs. EEE is most common in US Gulf Coast and Atlantic states. EEE is one of the more severe mosquito-transmitted diseases in the United States, but fortunately, it is a very rare disease in the United States ([\[link\]](#)).[\[footnote\]](#)[\[footnote\]](#) US Centers for Disease Control and Prevention, “Eastern Equine Encephalitis Virus Disease Cases and Deaths Reported to CDC by Year and

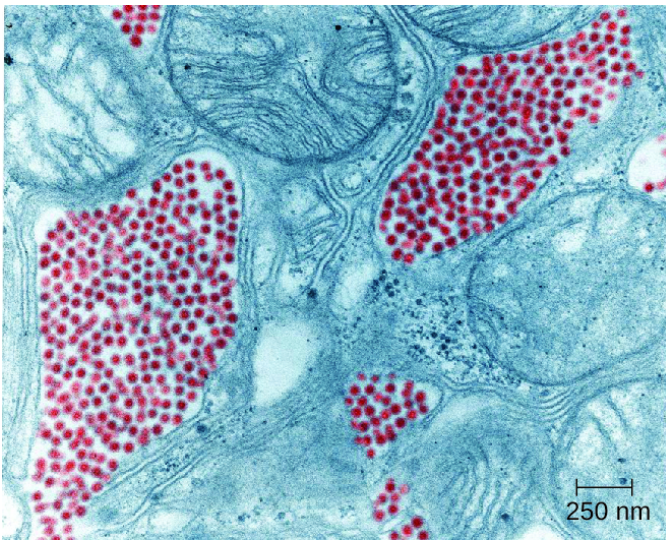
Clinical Presentation, 2004–2013,” 2014.

http://www.cdc.gov/EasternEquineEncephalitis/resources/EEEV-Cases-by-Year_2004-2013.pdf.

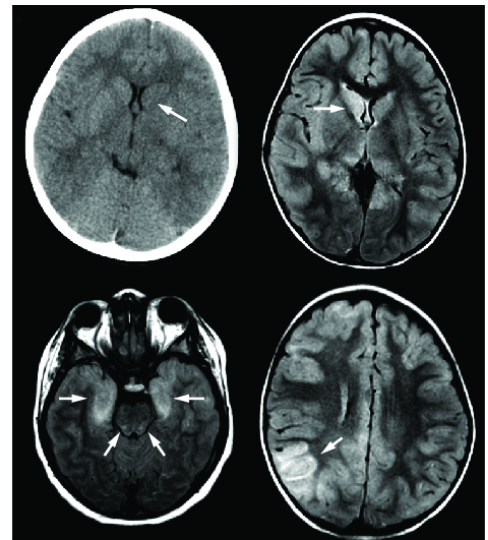
US Centers for Disease Control and Prevention, “Eastern Equine Encephalitis, Symptoms & Treatment, 2016,” Accessed June 29, 2016. <https://www.cdc.gov/easternequineencephalitis/tech/symptoms.html>.

Western equine encephalitis (WEE) is caused by western equine encephalitis virus (WEEV). WEEV is usually transmitted to horses and humans by the *Culex tarsalis* mosquitoes and, in the past decade, has caused very few cases of encephalitis in humans in the United States. In humans, WEE symptoms are less severe than EEE and include fever, chills, and vomiting, with a mortality rate of 3–4%. Like EEEV, birds are the natural reservoir for WEEV. Periodically, for indeterminate reasons, epidemics in human cases have occurred in North America in the past. The largest on record was in 1941, with more than 3400 cases. [\[footnote\]](#)

US Centers for Disease Control and Prevention, “Western Equine Encephalitis—United States and Canada, 1987,” *Morbidity and Mortality Weekly Report* 36, no. 39 (1987): 655.



(a)



(b)

(a) A false color TEM of a mosquito salivary gland cell shows an infection of the eastern equine encephalitis virus (red). (b) CT (left)

and MRI (right) scans of the brains of children with eastern equine encephalitis infections, showing abnormalities (arrows) resulting from the infection. (credit a, b: modifications of work by the Centers for Disease Control and Prevention)

St. Louis encephalitis (SLE), caused by St. Louis encephalitis virus (SLEV), is a rare form of encephalitis with symptoms occurring in fewer than 1% of infected patients. The natural reservoirs for SLEV are birds. SLEV is most often found in the Ohio-Mississippi River basin of the central United States and was named after a severe outbreak in Missouri in 1934. The worst outbreak of St. Louis encephalitis occurred in 1975, with over 2000 cases reported.[\[footnote\]](#) Humans become infected when bitten by *C. tarsalis*, *C. quinquefasciatus*, or *C. pipiens* mosquitoes carrying SLEV. Most patients are asymptomatic, but in a small number of individuals, symptoms range from mild flu-like syndromes to fatal encephalitis. The overall mortality rate for symptomatic patients is 5–15%.[\[footnote\]](#) US Centers for Disease Control and Prevention, “Saint Louis encephalitis, Epidemiology & Geographic Distribution,” Accessed June 30, 2016. <http://www.cdc.gov/sle/technical/epi.html>. US Centers for Disease Control and Prevention, “Saint Louis encephalitis, Symptoms and Treatment,” Accessed June 30, 2016. <http://www.cdc.gov/sle/technical/symptoms.html>.

Japanese encephalitis, caused by Japanese encephalitis virus (JEV), is the leading cause of vaccine-preventable encephalitis in humans and is endemic to some of the most populous countries in the world, including China, India, Japan, and all of Southeast Asia. JEV is transmitted to humans by *Culex* mosquitoes, usually the species *C. tritaeniorhynchus*. The biological reservoirs for JEV include pigs and wading birds. Most patients with JEV infections are asymptomatic, with symptoms occurring in fewer than 1% of infected individuals. However, about 25% of those who do develop encephalitis die, and among those who recover, 30–50% have psychiatric, neurologic, or cognitive impairment.[\[footnote\]](#) Fortunately, there is an effective vaccine that can prevent infection with JEV. The CDC

recommends this vaccine for travelers who expect to spend more than one month in endemic areas.

US Centers for Disease Control and Prevention, “Japanese Encephalitis, Symptoms and Treatment,” Accessed June 30, 2016.

<http://www.cdc.gov/japaneseencephalitis/symptoms/index.html>.

As the name suggests, West Nile virus (WNV) and its associated disease, **West Nile encephalitis (WNE)**, did not originate in North America. Until 1999, it was endemic in the Middle East, Africa, and Asia; however, the first US cases were identified in New York in 1999, and by 2004, the virus had spread across the entire continental United States. Over 35,000 cases, including 1400 deaths, were confirmed in the five-year period between 1999 and 2004. WNV infection remains reportable to the CDC.

WNV is transmitted to humans by *Culex* mosquitoes from its natural reservoir, infected birds, with 70–80% of infected patients experiencing no symptoms. Most symptomatic cases involve only mild, flu-like symptoms, but fewer than 1% of infected people develop severe and sometimes fatal encephalitis or meningitis. The mortality rate in WNV patients who develop neurological disease is about 10%. More information about West Nile virus can be found in [Modes of Disease Transmission](#).

Note:

This [interactive map](#) identifies cases of several arboviral diseases in humans and reservoir species by state and year for the United States.

Note:

- Why is it unlikely that arboviral encephalitis viruses will be eradicated in the future?
- Which is the most common form of viral encephalitis in the United States?

Note:**Part 2**

Levofloxacin is a quinolone antibiotic that is often prescribed to treat bacterial infections of the respiratory tract, including pneumonia and bronchitis. But after taking the medication for a week, David returned to his physician sicker than before. He claimed that the antibiotic had no effect on his earlier symptoms. In addition, he now was experiencing headaches, a stiff neck, and difficulty focusing at work. He also showed the doctor a rash that had developed on his arms over the past week. His doctor, more concerned now, began to ask about David's activities over the past two weeks.

David explained that he had been recently working on a project to disassemble an old barn. His doctor collected sputum samples and scrapings from David's rash for cultures. A spinal tap was also performed to examine David's CSF. Microscopic examination of his CSF revealed encapsulated yeast cells. Based on this result, the doctor prescribed a new antimicrobial therapy using amphotericin B and flucytosine.

- Why was the original treatment ineffective?
- Why is the presence of a capsule clinically important?

Jump to the [previous](#) Clinical Focus box. Jump to the [next](#) Clinical Focus box.

Zika Virus Infection

Zika virus infection is an emerging arboviral disease associated with human illness in Africa, Southeast Asia, and South and Central America; however, its range is expanding as a result of the widespread range of its mosquito vector. The first cases originating in the United States were reported in 2016. The Zika virus was initially described in 1947 from monkeys in the Zika Forest of Uganda through a network that monitored yellow fever. It was not considered a serious human pathogen until the first large-scale outbreaks occurred in Micronesia in 2007; [\[footnote\]](#) however, the virus has gained notoriety over the past decade, as it has emerged as a cause of

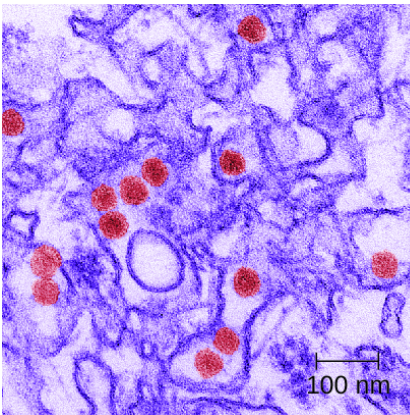
symptoms similar to other arboviral infections that include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache. Mosquitoes of the *Aedes* genus are the primary vectors, although the virus can also be transmitted sexually, from mother to baby during pregnancy, or through a blood transfusion.

Sikka, Veronica, Vijay Kumar Chattu, Raaj K. Popli, Sagar C. Galwankar, Dhanashree Kelkar, Stanley G. Sawicki, Stanislaw P. Stawicki, and Thomas J. Papadimos, “The Emergence of Zika Virus as a Global Health Security Threat: A Review and a Consensus Statement of the INDUSEM Joint Working Group (JWG),” *Journal of Global Infectious Diseases* 8, no. 1 (2016): 3.

Most Zika virus infections result in mild symptoms such as fever, a slight rash, or conjunctivitis. However, infections in pregnant women can adversely affect the developing fetus. Reports in 2015 indicate fetal infections can result in brain damage, including a serious birth defect called microcephaly, in which the infant is born with an abnormally small head ([\[link\]](#)).[\[footnote\]](#)

Mlakar, Jernej, Misa Korva, Nataša Tul, Mara Popović, Mateja Poljšak-Prijatelj, Jerica Mraz, Marko Kolenc et al., “Zika Virus Associated with Microcephaly,” *New England Journal of Medicine* 374, no. 10 (2016): 951-8.

Diagnosis of Zika is primarily based on clinical symptoms. However, the FDA recently authorized the use of a Zika virus RNA assay, Trioplex RT-PCR, and Zika MAC-ELISA to test patient blood and urine to confirm Zika virus disease. There are currently no antiviral treatments or vaccines for Zika virus, and treatment is limited to supportive care.



(a)



infant with microcephaly



normal infant

(b)

(a) This colorized electron micrograph shows Zika virus particles (red). (b) Women infected by the Zika virus during pregnancy may give birth to children with microcephaly, a deformity characterized by an abnormally small head and brain. (credit a, b: modifications of work by the Centers for Disease Control and Prevention)

Note:

- What are the signs and symptoms of Zika virus infection in adults?
- Why is Zika virus infection considered a serious public health threat?

Rabies

Rabies is a deadly zoonotic disease that has been known since antiquity. The disease is caused by rabies virus (RV), a member of the family Rhabdoviridae, and is primarily transmitted through the bite of an infected mammal. Rhabdoviridae are enveloped RNA viruses that have a distinctive bullet shape ([\[link\]](#)); they were first studied by Louis Pasteur, who obtained rabies virus from rabid dogs and cultivated the virus in rabbits. He

successfully prepared a rabies vaccine using dried nerve tissues from infected animals. This vaccine was used to first treat an infected human in 1885.

The most common reservoirs in the United States are wild animals such as raccoons (30.2% of all animal cases during 2014), bats (29.1%), skunks (26.3%), and foxes (4.1%); collectively, these animals were responsible for a total of 92.6% of animal rabies cases in the United States in 2014. The remaining 7.4% of cases that year were in domesticated animals such as dogs, cats, horses, mules, sheep, goats, and llamas.[\[footnote\]](#) While there are typically only one or two human cases per year in the United States, rabies still causes tens of thousands of human deaths per year worldwide, primarily in Asia and Africa.

US Centers for Disease Control and Prevention, “Rabies, Wild Animals,” 2016. Accessed September 13, 2016.

http://www.cdc.gov/rabies/location/usa/surveillance/wild_animals.html.

The low incidence of rabies in the United States is primarily a result of the widespread vaccination of dogs and cats. An oral vaccine is also used to protect wild animals, such as raccoons and foxes, from infection. Oral vaccine programs tend to focus on geographic areas where rabies is endemic.[\[footnote\]](#) The oral vaccine is usually delivered in a package of bait that is dropped by airplane, although baiting in urban areas is done by hand to maximize safety.[\[footnote\]](#) Many countries require a quarantine or proof of rabies vaccination for domestic pets being brought into the country. These procedures are especially strict in island nations where rabies is not yet present, such as Australia.

Slate, Dennis, Charles E. Rupprecht, Jane A. Rooney, Dennis Donovan, Donald H. Lein, and Richard B. Chipman, “Status of Oral Rabies Vaccination in Wild Carnivores in the United States,” *Virus Research* 111, no. 1 (2005): 68-76.

Finnegan, Christopher J., Sharon M. Brookes, Nicholas Johnson, Jemma Smith, Karen L. Mansfield, Victoria L. Keene, Lorraine M. McElhinney, and Anthony R. Fooks, “Rabies in North America and Europe,” *Journal of the Royal Society of Medicine* 95, no. 1 (2002): 9-13.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1279140/>.

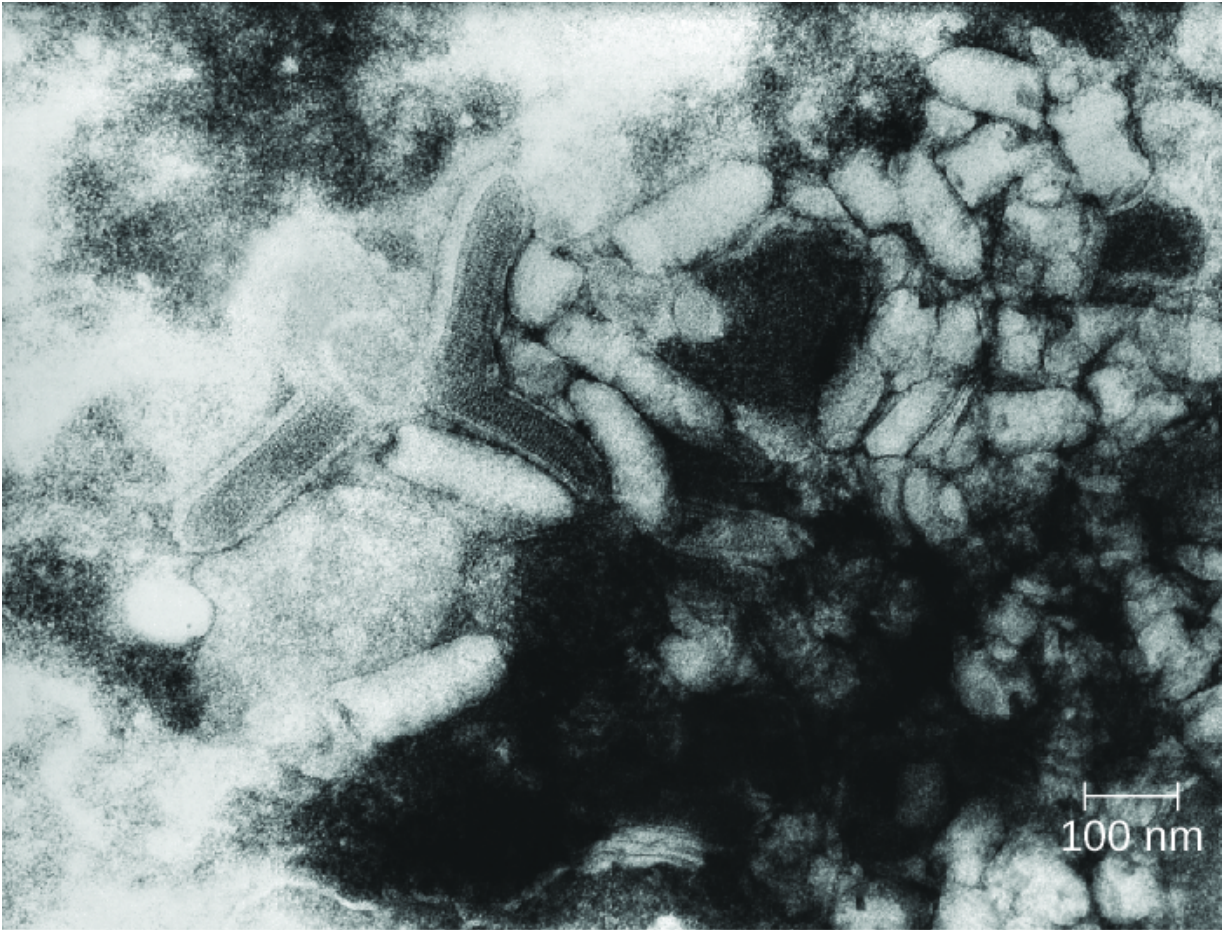
The incubation period for rabies can be lengthy, ranging from several weeks or months to over a year. As the virus replicates, it moves from the site of the bite into motor and sensory axons of peripheral nerves and spreads from nerve to nerve using a process called retrograde transport, eventually making its way to the CNS through the spinal ganglia. Once rabies virus reaches the brain, the infection leads to encephalitis caused by the disruption of normal neurotransmitter function, resulting in the symptoms associated with rabies. The virions act in the synaptic spaces as competitors with a variety of neurotransmitters for acetylcholine, GABA, and glycine receptors. Thus, the action of rabies virus is neurotoxic rather than cytotoxic. After the rabies virus infects the brain, it can continue to spread through other neuronal pathways, traveling out of the CNS to tissues such as the salivary glands, where the virus can be released. As a result, as the disease progresses the virus can be found in many other tissues, including the salivary glands, taste buds, nasal cavity, and tears.

The early symptoms of rabies include discomfort at the site of the bite, fever, and headache. Once the virus reaches the brain and later symptoms appear, the disease is always fatal. Terminal rabies cases can end in one of two ways: either furious or paralytic rabies. Individuals with furious rabies become very agitated and hyperactive. Hydrophobia (a fear of water) is common in patients with furious rabies, which is caused by muscular spasms in the throat when swallowing or thinking about water. Excess salivation and a desire to bite can lead to foaming of the mouth. These behaviors serve to enhance the likelihood of viral transmission, although contact with infected secretions like saliva or tears alone is sufficient for infection. The disease culminates after just a few days with terror and confusion, followed by cardiovascular and respiratory arrest. In contrast, individuals with paralytic rabies generally follow a longer course of disease. The muscles at the site of infection become paralyzed. Over a period of time, the paralysis slowly spreads throughout the body. This paralytic form of disease culminates in coma and death.

Before present-day diagnostic methods were available, rabies diagnosis was made using a clinical case history and histopathological examination of biopsy or autopsy tissues, looking for the presence of Negri bodies. We now know these histologic changes *cannot* be used to confirm a rabies diagnosis.

There are no tests that can detect rabies virus in humans at the time of the bite or shortly thereafter. Once the virus has begun to replicate (but before clinical symptoms occur), the virus can be detected using an immunofluorescence test on cutaneous nerves found at the base of hair follicles. Saliva can also be tested for viral genetic material by reverse transcription followed by polymerase chain reaction (RT-PCR). Even when these tests are performed, most suspected infections are treated as positive in the absence of contravening evidence. It is better that patients undergo unnecessary therapy because of a false-positive result, rather than die as the result of a false-negative result.

Human rabies infections are treated by immunization with multiple doses of an attenuated vaccine to develop active immunity in the patient (see the Clinical Focus feature in the chapter on [Acellular Pathogens](#)). Vaccination of an already-infected individual has the potential to work because of the slow progress of the disease, which allows time for the patient's immune system to develop antibodies against the virus. Patients may also be treated with human rabies immune globulin (antibodies to the rabies virus) to encourage passive immunity. These antibodies will neutralize any free viral particles. Although the rabies infection progresses slowly in peripheral tissues, patients are not normally able to mount a protective immune response on their own.



Virions of the rabies virus have a characteristic bullet-like shape.
(credit: modification of work by the Centers for Disease Control and Prevention)

Note:

- How does the bite from an infected animal transmit rabies?
- What is the goal of wildlife vaccination programs for rabies?
- How is rabies treated in a human?

Poliomyelitis

Poliomyelitis (polio), caused by poliovirus, is a primarily intestinal disease that, in a small percentage of cases, proceeds to the nervous system, causing paralysis and, potentially, death. Poliovirus is highly contagious, with transmission occurring by the fecal-oral route or by aerosol or droplet transmission. Approximately 72% of all poliovirus infections are asymptomatic; another 25% result only in mild intestinal disease, producing nausea, fever, and headache.[\[footnote\]](#) However, even in the absence of symptoms, patients infected with the virus can shed it in feces and oral secretions, potentially transmitting the virus to others. In about one case in every 200, the poliovirus affects cells in the CNS.[\[footnote\]](#)

US Centers for Disease Control and Prevention, “Global Health – Polio,” 2014. Accessed June 30, 2016. <http://www.cdc.gov/polio/about/index.htm>.
US Centers for Disease Control and Prevention, “Global Health – Polio,” 2014. Accessed June 30, 2016. <http://www.cdc.gov/polio/about/index.htm>.

After it enters through the mouth, initial replication of poliovirus occurs at the site of implantation in the pharynx and gastrointestinal tract. As the infection progresses, poliovirus is usually present in the throat and in the stool before the onset of symptoms. One week after the onset of symptoms, there is less poliovirus in the throat, but for several weeks, poliovirus continues to be excreted in the stool. Poliovirus invades local lymphoid tissue, enters the bloodstream, and then may infect cells of the CNS.

Replication of poliovirus in motor neurons of the anterior horn cells in the spinal cord, brain stem, or motor cortex results in cell destruction and leads to flaccid paralysis. In severe cases, this can involve the respiratory system, leading to death. Patients with impaired respiratory function are treated using positive-pressure ventilation systems. In the past, patients were sometimes confined to Emerson respirators, also known as iron lungs ([link](#)).

Direct detection of the poliovirus from the throat or feces can be achieved using reverse transcriptase PCR (RT-PCR) or genomic sequencing to identify the genotype of the poliovirus infecting the patient. Serological tests can be used to determine whether the patient has been previously vaccinated. There are no therapeutic measures for polio; treatment is limited

to various supportive measures. These include pain relievers, rest, heat therapy to ease muscle spasms, physical therapy and corrective braces if necessary to help with walking, and mechanical ventilation to assist with breathing if necessary.



(a)



(b)

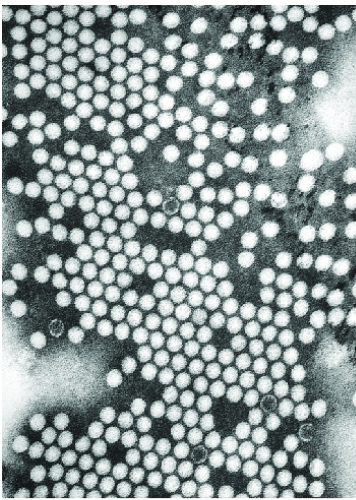
(a) An Emerson respiratory (or iron lung) that was used to help some polio victims to breathe. (b) Polio can also result in impaired motor function. (credit b: modification of work by the Centers for Disease Control and Prevention)

Two different vaccines were introduced in the 1950s that have led to the dramatic decrease in polio worldwide ([link](#)). The Salk vaccine is an inactivated polio virus that was first introduced in 1955. This vaccine is delivered by intramuscular injection. The Sabin vaccine is an oral polio vaccine that contains an attenuated virus; it was licensed for use in 1962. There are three serotypes of poliovirus that cause disease in humans; both the Salk and the Sabin vaccines are effective against all three.

Attenuated viruses from the Sabin vaccine are shed in the feces of immunized individuals and thus have the potential to infect nonimmunized

individuals. By the late 1990s, the few polio cases originating in the United States could be traced back to the Sabin vaccine. In these cases, mutations of the attenuated virus following vaccination likely allowed the microbe to revert to a virulent form. For this reason, the United States switched exclusively to the Salk vaccine in 2000. Because the Salk vaccine contains an inactivated virus, there is no risk of transmission to others (see [Vaccines](#)). Currently four doses of the vaccine are recommended for children: at 2, 4, and 6–18 months of age, and at 4–6 years of age.

In 1988, WHO launched the Global Polio Eradication Initiative with the goal of eradicating polio worldwide through immunization. That goal is now close to being realized. Polio is now endemic in only a few countries, including Afghanistan, Pakistan, and Nigeria, where vaccination efforts have been disrupted by military conflict or political instability.



(a)



(b)

(a) Polio is caused by the poliovirus. (b) Two American virologists developed the first polio vaccines: Albert Sabin (left) and Jonas Salk (right). (credit a: modification of work by the Centers for Disease Control and Prevention)

Note:**The Terror of Polio**

In the years after World War II, the United States and the Soviet Union entered a period known as the Cold War. Although there was no armed conflict, the two super powers were diplomatically and economically isolated from each other, as represented by the so-called Iron Curtain between the Soviet Union and the rest of the world. After 1950, migration or travel outside of the Soviet Union was exceedingly difficult, and it was equally difficult for foreigners to enter the Soviet Union. The United States also placed strict limits on Soviets entering the country. During the Eisenhower administration, only 20 graduate students from the Soviet Union were allowed to come to study in the United States per year.

Yet even the Iron Curtain was no match for polio. The Salk vaccine became widely available in the West in 1955, and by the time the Sabin vaccine was ready for clinical trials, most of the susceptible population in the United States and Canada had already been vaccinated against polio. Sabin needed to look elsewhere for study participants. At the height of the Cold War, Mikhail Chumakov was allowed to come to the United States to study Sabin's work. Likewise, Sabin, an American microbiologist, was allowed to travel to the Soviet Union to begin clinical trials. Chumakov organized Soviet-based production and managed the experimental trials to test the new vaccine in the Soviet Union. By 1959, over ten million Soviet children had been safely treated with Sabin's vaccine.

As a result of a global vaccination campaign with the Sabin vaccine, the overall incidence of polio has dropped dramatically. Today, polio has been nearly eliminated around the world and is only rarely seen in the United States. Perhaps one day soon, polio will become the third microbial disease to be eradicated from the general population [small pox and rinderpest (the cause of cattle plague) being the first two].

Note:

- How is poliovirus transmitted?
- Compare the pros and cons of each of the two polio vaccines.

Transmissible Spongiform Encephalopathies

Acellular infectious agents called prions are responsible for a group of related diseases known as transmissible spongiform encephalopathies (TSEs) that occurs in humans and other animals (see [Viroids](#), [Virusoids](#), and [Prions](#)). All TSEs are degenerative, fatal neurological diseases that occur when brain tissue becomes infected by prions. These diseases have a slow onset; symptoms may not become apparent until after an incubation period of years and perhaps decades, but death usually occurs within months to a few years after the first symptoms appear.

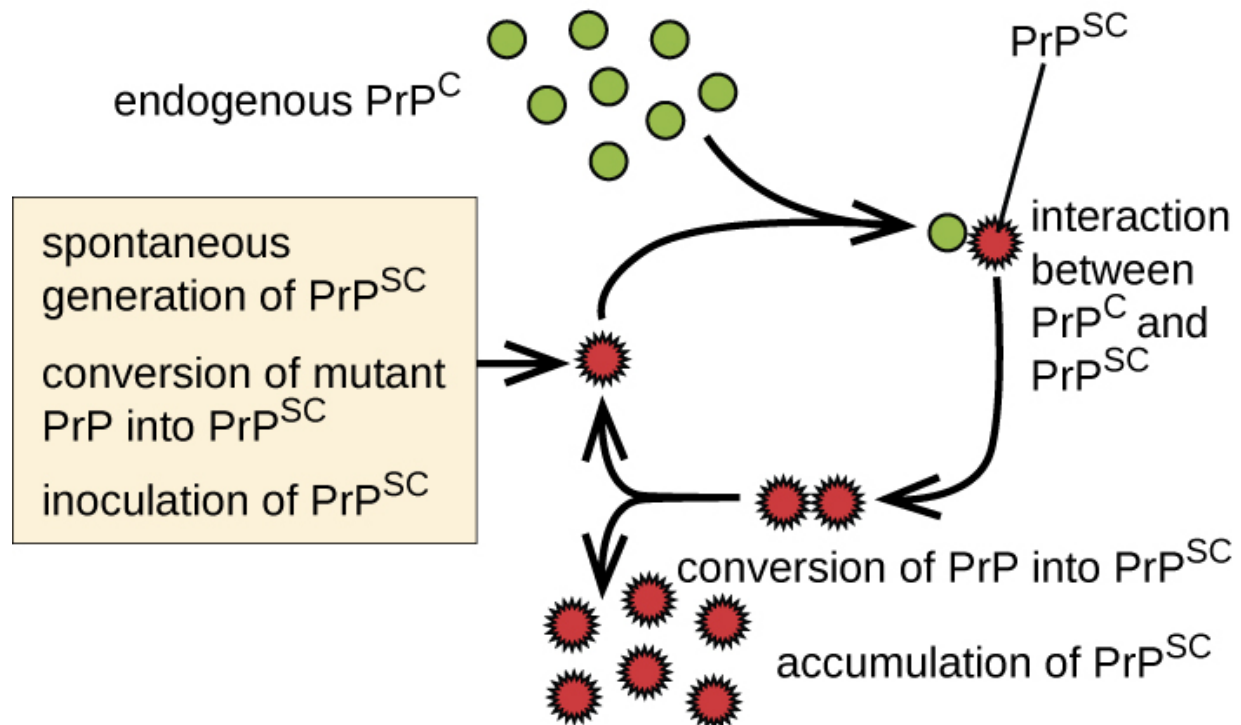
TSEs in animals include **scrapie**, a disease in sheep that has been known since the 1700s, and **chronic wasting disease**, a disease of deer and elk in the United States and Canada. **Mad cow disease** is seen in cattle and can be transmitted to humans through the consumption of infected nerve tissues. Human prion diseases include **Creutzfeldt-Jakob disease** and **kuru**, a rare disease endemic to Papua New Guinea.

Prions are infectious proteinaceous particles that are not viruses and do not contain nucleic acid. They are typically transmitted by exposure to and ingestion of infected nervous system tissues, tissue transplants, blood transfusions, or contaminated fomites. Prion proteins are normally found in a healthy brain tissue in a form called PrP^{C} . However, if this protein is misfolded into a denatured form (PrP^{Sc}), it can cause disease. Although the exact function of PrP^{C} is not currently understood, the protein folds into mostly alpha helices and binds copper. The rogue protein, on the other hand, folds predominantly into beta-pleated sheets and is resistant to proteolysis. In addition, PrP^{Sc} can induce PrP^{C} to become misfolded and produce more rogue protein ([link](#)).

As PrP^{Sc} accumulates, it aggregates and forms fibrils within nerve cells. These protein complexes ultimately cause the cells to die. As a consequence, brain tissues of infected individuals form masses of neurofibrillary tangles and amyloid plaques that give the brain a spongy appearance, which is why these diseases are called spongiform encephalopathy ([link](#)). Damage to brain tissue results in a variety of neurological symptoms. Most commonly, affected individuals suffer from

memory loss, personality changes, blurred vision, uncoordinated movements, and insomnia. These symptoms gradually worsen over time and culminate in coma and death.

The gold standard for diagnosing TSE is the histological examination of brain biopsies for the presence of characteristic amyloid plaques, vacuoles, and prion proteins. Great care must be taken by clinicians when handling suspected prion-infected materials to avoid becoming infected themselves. Other tissue assays search for the presence of the 14-3-3 protein, a marker for prion diseases like Creutzfeldt-Jakob disease. New assays, like RT-QuIC (real-time quaking-induced conversion), offer new hope to effectively detect the abnormal prion proteins in tissues earlier in the course of infection. Prion diseases cannot be cured. However, some medications may help slow their progress. Medical support is focused on keeping patients as comfortable as possible despite progressive and debilitating symptoms.



The replicative cycle of misfolded prion proteins.

Note:

Because prion-contaminated materials are potential sources of infection for clinical scientists and physicians, both the [World Health Organization](#) and [CDC](#) provide information to inform, educate and minimize the risk of infections due to prions.

Note:

- Do prions reproduce in the conventional sense?
- What is the connection between prions and the removal of animal byproducts from the food of farm animals?

Note:**Acellular Infections of the Nervous System**

Serious consequences are the common thread among these neurological diseases. Several cause debilitating paralysis, and some, such as Creutzfeldt-Jakob disease and rabies, are always or nearly always fatal. Since few drugs are available to combat these infections, vector control and vaccination are critical for prevention and containment. [\[link\]](#) summarizes some important viral and prion infections of the nervous system.

Acellular Infections of the Nervous System						
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Arboviral encephalitis (eastern equine, western equine, St. Louis, West Nile, Japanese)	EEEV, WEEV, SLEV, WNV, JEV	In mild cases, fever, chills, headaches, and restlessness; in serious cases, encephalitis leading to convulsions, coma, and death	From bird reservoirs to humans (and horses) by mosquito vectors of various species	Serologic testing of serum or CSF	None	Human vaccine available for JEV only; no vaccines available for other arboviruses
Creutzfeldt-Jacob Disease and other TSEs	Prions	Memory loss, confusion, blurred vision, uncoordinated movement, insomnia, coma, death	Exposure to infected nerve tissue via consumption or transplant, inherited	Tissue biopsy	None	None
Poliomyelitis	Poliovirus	Asymptomatic or mild nausea, fever, headache in most cases; in neurological infections, flaccid paralysis and potentially fatal respiratory paralysis	Fecal-oral route or contact with droplets or aerosols	Culture of poliovirus, PCR	None	Attenuated vaccine (Sabin), killed vaccine (Salk)
Rabies	Rabies virus (RV)	Fever, headaches, hyperactivity, hydrophobia, excessive salivation, terrors, confusion, spreading paralysis, coma, always fatal if not promptly treated	From bite of infected mammal	Viral antigen in tissue, antibodies to virus	Attenuated vaccine, rabies immunoglobulin	Attenuated vaccine
Viral meningitis	HSV-1, HSV-2, varicella zoster virus, mumps virus, influenza virus, measles virus	Nausea, vomiting, photophobia, stiff neck, confusion, symptoms generally resolve within 7–10 days	Sequela of primary viral infection	Testing of oral, fecal, blood, or CSF samples	Varies depending on cause	Varies depending on cause
Zika virus infection	Zika virus	Fever, rash, conjunctivitis; in pregnant women, can cause fetal brain damage and microcephaly	Between humans by <i>Aedes</i> spp. mosquito vectors, also may be transmitted sexually or via blood transfusion	Zika virus RNA assay, Trioplex RT-PCR, Zika MAC-ELISA test	None	None

Key Concepts and Summary

- **Viral meningitis** is more common and generally less severe than bacterial meningitis. It can result from secondary sequelae of many viruses or be caused by infections of arboviruses.
- Various types of **arboviral encephalitis** are concentrated in particular geographic locations throughout the world. These mosquito-borne viral infections of the nervous system are typically mild, but they can be life-threatening in some cases.
- **Zika virus** is an emerging arboviral infection with generally mild symptoms in most individuals, but infections of pregnant women can cause the birth defect microcephaly.
- **Polio** is typically a mild intestinal infection but can be damaging or fatal if it progresses to a neurological disease.
- **Rabies** is nearly always fatal when untreated and remains a significant problem worldwide.
- **Transmissible spongiform encephalopathies** such as **Creutzfeldt-Jakob disease** and **kuru** are caused by prions. These diseases are untreatable and ultimately fatal. Similar prion diseases are found in animals.

Multiple Choice

Exercise:

Problem:

Which of these diseases can be prevented with a vaccine for humans?

- a. eastern equine encephalitis
- b. western equine encephalitis
- c. West Nile encephalitis
- d. Japanese encephalitis

Solution:

D

Exercise:

Problem:

Which of these diseases does NOT require the introduction of foreign nucleic acid?

- a. kuru
- b. polio
- c. rabies
- d. St. Louis encephalitis

Solution:

A

Exercise:

Problem:

Which of these is true of the Sabin but NOT the Salk polio vaccine?

- a. requires four injections
- b. currently administered in the United States
- c. mimics the normal route of infection
- d. is an inactivated vaccine

Solution:

C

Exercise:

Problem:

Which of the following animals is NOT a typical reservoir for the spread of rabies?

- a. dog

- b. bat
- c. skunk
- d. chicken

Solution:

D

Fill in the Blank

Exercise:

Problem: The rogue form of the prion protein is called _____.

Solution:

PrP^{Sc}

Exercise:

Problem:

_____ are the most common reservoir for the rabies virus worldwide.

Solution:

Dogs

Exercise:

Problem:

_____ was the scientist who developed the inactivated polio vaccine.

Solution:

Jonas Salk

Exercise:

Problem: _____ is a prion disease of deer and elk.

Solution:

Chronic wasting disease

Exercise:

Problem:

The rogue form of prion protein exists primarily in the _____ conformation.

Solution:

beta sheet

Short Answer

Exercise:

Problem:

Explain how a person could contract variant Creutzfeldt-Jakob disease by consuming products from a cow with bovine spongiform encephalopathy (mad cow disease).

Critical Thinking

Exercise:

Problem:

If the Sabin vaccine is being used to eliminate polio worldwide, explain why a country with a near zero infection rate would opt to use the Salk vaccine but not the Sabin vaccine?

Fungal and Parasitic Diseases of the Nervous System

LEARNING OBJECTIVES

- Identify the most common fungi that can cause infections of the nervous system
- Compare the major characteristics of specific fungal diseases affecting the nervous system

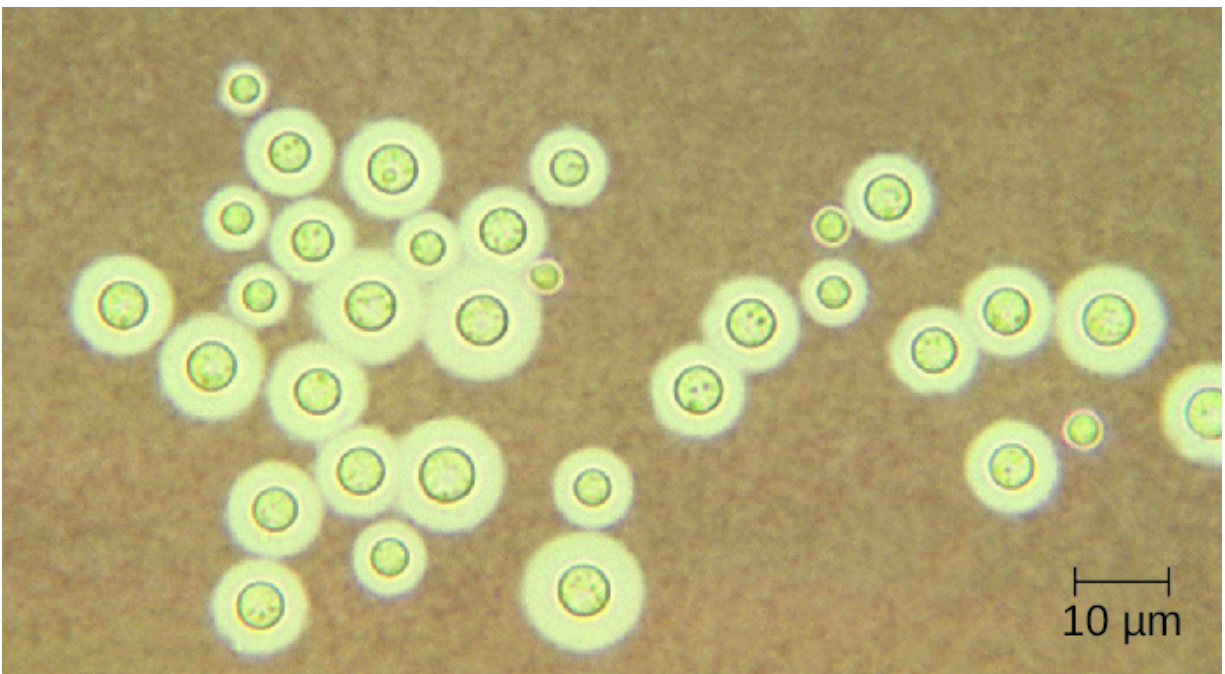
Fungal infections of the nervous system, called **neuromycoses**, are rare in healthy individuals. However, neuromycoses can be devastating in immunocompromised or elderly patients. Several eukaryotic parasites are also capable of infecting the nervous system of human hosts. Although relatively uncommon, these infections can also be life-threatening in immunocompromised individuals. In this section, we will first discuss neuromycoses, followed by parasitic infections of the nervous system.

Cryptococcal Meningitis

Cryptococcus neoformans is a fungal pathogen that can cause meningitis. This yeast is commonly found in soils and is particularly associated with pigeon droppings. It has a thick capsule that serves as an important virulence factor, inhibiting clearance by phagocytosis. Most *C. neoformans* cases result in subclinical respiratory infections that, in healthy individuals, generally resolve spontaneously with no long-term consequences (see [Respiratory Mycoses](#)). In immunocompromised patients or those with other underlying illnesses, the infection can progress to cause meningitis and granuloma formation in brain tissues. *Cryptococcus* antigens can also serve to inhibit cell-mediated immunity and delayed-type hypersensitivity.

Cryptococcus can be easily cultured in the laboratory and identified based on its extensive capsule ([\[link\]](#)). *C. neoformans* is frequently cultured from urine samples of patients with disseminated infections.

Prolonged treatment with antifungal drugs is required to treat cryptococcal infections. Combined therapy is required with amphotericin B plus flucytosine for at least 10 weeks. Many antifungal drugs have difficulty crossing the blood-brain barrier and have strong side effects that necessitate low doses; these factors contribute to the lengthy time of treatment. Patients with AIDS are particularly susceptible to *Cryptococcus* infections because of their compromised immune state. AIDS patients with cryptococcosis can also be treated with antifungal drugs, but they often have relapses; lifelong doses of fluconazole may be necessary to prevent reinfection.



An India ink-negative stain of *C. neoformans* showing the thick capsules around the spherical yeast cells. (credit: modification of work by Centers for Disease Control and Prevention)

Note:

- Why are neuromycoses infections rare in the general population?
- How is a cryptococcal infection acquired?

Note:**Neuromycoses**

Neuromycoses typically occur only in immunocompromised individuals and usually only invade the nervous system after first infecting a different body system. As such, many diseases that sometimes affect the nervous system have already been discussed in previous chapters. [\[link\]](#) presents some of the most common fungal infections associated with neurological disease. This table includes only the neurological aspects associated with these diseases; it does not include characteristics associated with other body systems.

Neuromycoses					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Aspergillosis	<i>Aspergillus fumigatus</i>	Meningitis, brain abscesses	Dissemination from respiratory infection	CSF, routine culture	Amphotericin B, voriconazole
Candidiasis	<i>Candida albicans</i>	Meningitis	Oropharynx or urogenital	CSF, routine culture	Amphotericin B, flucytosine
Coccidioidomycosis (Valley fever)	<i>Coccidioides immitis</i>	Meningitis (in about 1% of infections)	Dissemination from respiratory infection	CSF, routine culture	Amphotericin B, azoles
Cryptococcosis	<i>Cryptococcus neoformans</i>	Meningitis, granuloma formation in brain	Inhalation	Negative stain of CSF, routine culture	Amphotericin B, flucytosine
Histoplasmosis	<i>Histoplasma capsulatum</i>	Meningitis, granulomas in the brain	Dissemination from respiratory infection	CSF, routine culture	Amphotericin B, itraconazole
Mucormycosis	<i>Rhizopus arrhizus</i>	Brain abscess	Nasopharynx	CSF, routine culture	Amphotericin B, azoles

Note:

Resolution

David's new prescription for two antifungal drugs, amphotericin B and flucytosine, proved effective, and his condition began to improve. Culture results from David's sputum, skin, and CSF samples confirmed a fungal infection. All were positive for *C. neoformans*. Serological tests of his tissues were also positive for the *C. neoformans* capsular polysaccharide antigen.

Since *C. neoformans* is known to occur in bird droppings, it is likely that David had been exposed to the fungus while working on the barn. Despite this exposure, David's doctor explained to him that immunocompetent people rarely contract cryptococcal meningitis and that his immune system had likely been compromised by the anti-inflammatory medication he was taking to treat his Crohn's disease. However, to rule out other possible causes of immunodeficiency, David's doctor recommended that he be tested for HIV.

After David tested negative for HIV, his doctor took him off the corticosteroid he was using to manage his Crohn's disease, replacing it with a different class of drug. After several weeks of antifungal treatments, David managed a full recovery.

Jump to the [previous](#) Clinical Focus box.

Amoebic Meningitis

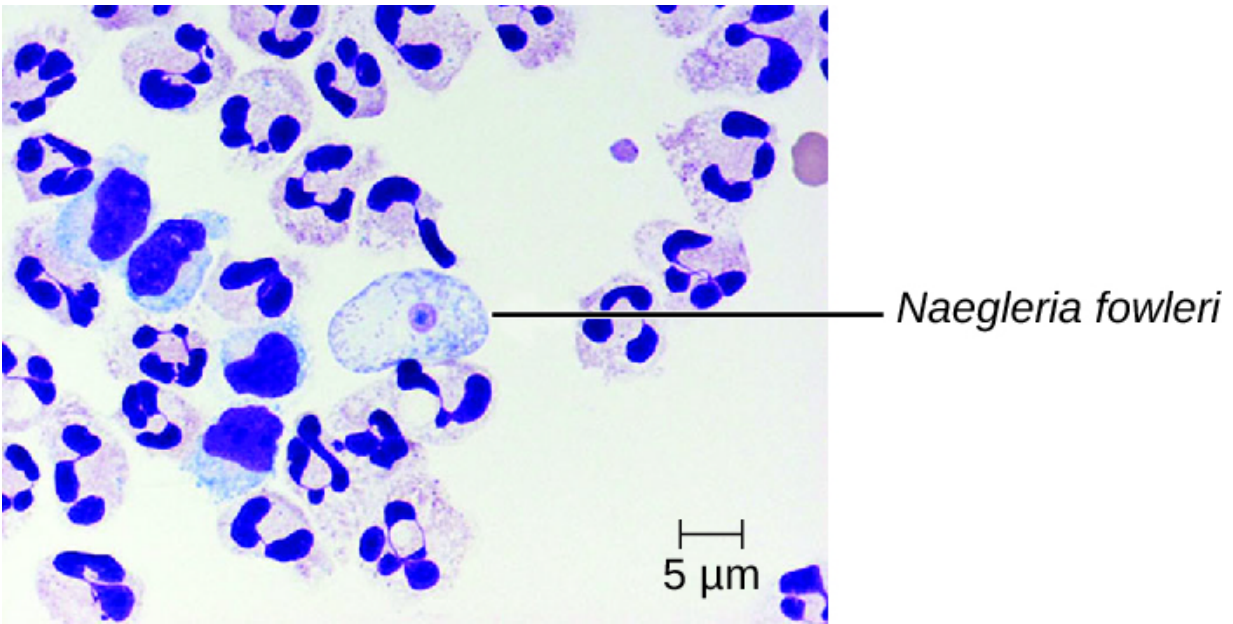
Primary amoebic meningoencephalitis (PAM) is caused by *Naegleria fowleri*. This amoeboflagellate is commonly found free-living in soils and water. It can exist in one of three forms—the infective amoebic trophozoite form, a motile flagellate form, and a resting cyst form. PAM is a rare disease that has been associated with young and otherwise healthy individuals. Individuals are typically infected by the amoeba while swimming in warm bodies of freshwater such as rivers, lakes, and hot springs. The pathogenic trophozoite infects the brain by initially entering through nasal passages to the sinuses; it then moves down olfactory nerve fibers to penetrate the submucosal nervous plexus, invades the cribriform plate, and reaches the subarachnoid space. The subarachnoid space is highly vascularized and is a route of dissemination of trophozoites to other areas of

the CNS, including the brain ([\[link\]](#)). Inflammation and destruction of gray matter leads to severe headaches and fever. Within days, confusion and convulsions occur and quickly progress to seizures, coma, and death. The progression can be very rapid, and the disease is often not diagnosed until autopsy.

N. fowleri infections can be confirmed by direct observation of CSF; the amoebae can often be seen moving while viewing a fresh CSF wet mount through a microscope. Flagellated forms can occasionally also be found in CSF. The amoebae can be stained with several stains for identification, including Giemsa-Wright or a modified trichrome stain. Detection of antigens with indirect immunofluorescence, or genetic analysis with PCR, can be used to confirm an initial diagnosis. *N. fowleri* infections are nearly always fatal; only 3 of 138 patients with PAM in the United States have survived.[\[footnote\]](#) A new experimental drug called miltefosine shows some promise for treating these infections. This drug is a phosphatidylcholine derivative that is thought to inhibit membrane function in *N. fowleri*, triggering apoptosis and disturbance of lipid-dependent cell signaling pathways.[\[footnote\]](#) When administered early in infection and coupled with therapeutic hypothermia (lowering the body's core temperature to reduce the cerebral edema associated with infection), this drug has been successfully used to treat primary amoebic encephalitis. US Centers for Disease Control and Prevention, "*Naegleria fowleri*—Primary Amoebic Meningoencephalitis (PAM)—Amebic Encephalitis," 2016. Accessed June 30, 2016.

<http://www.cdc.gov/parasites/naegleria/treatment.html>.

Dorlo, Thomas PC, Manica Balasegaram, Jos H. Beijnen, and Peter J. de Vries, "Miltefosine: A Review of Its Pharmacology and Therapeutic Efficacy in the Treatment of Leishmaniasis," *Journal of Antimicrobial Chemotherapy* 67, no. 11 (2012): 2576-97.



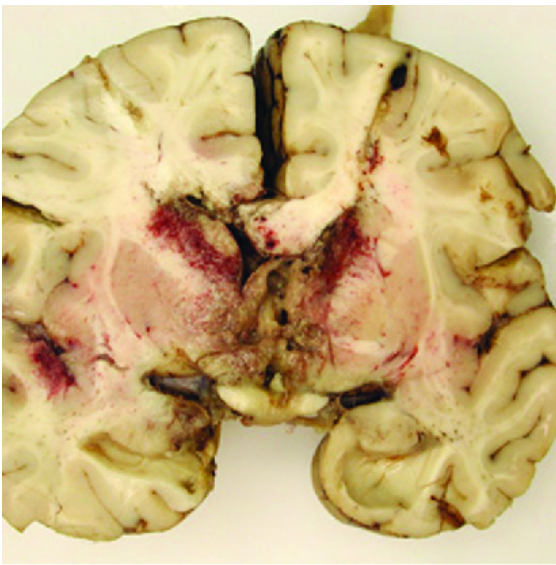
Free-living amoeba in human brain tissue from a patient suffering from PAM. (credit: modification of work by the Centers for Disease Control and Prevention)

Granulomatous Amoebic Encephalitis

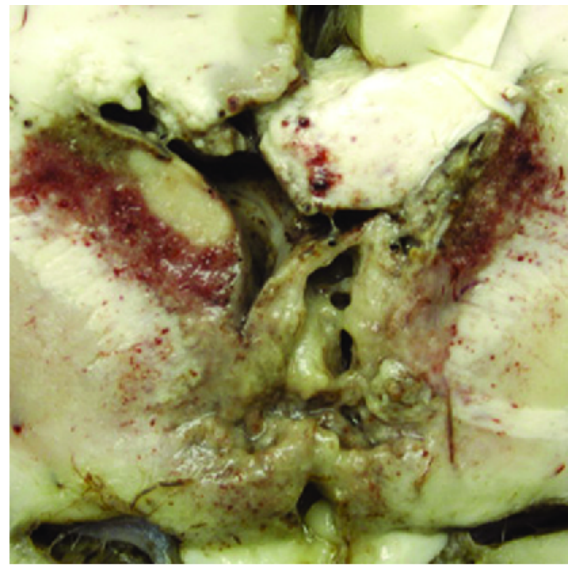
Acanthamoeba and *Balamuthia* species are free-living amoebae found in many bodies of fresh water. Human infections by these amoebae are rare. However, they can cause amoebic keratitis in contact lens wearers (see [Protozoan and Helminthic Infections of the Eyes](#)), disseminated infections in immunocompromised patients, and **granulomatous amoebic encephalitis (GAE)** in severe cases. Compared to PAM, GAE tend to be subacute infections. The microbe is thought to enter through either the nasal sinuses or breaks in the skin. It is disseminated hematogenously and can invade the CNS. There, the infections lead to inflammation, formation of lesions, and development of typical neurological symptoms of encephalitis ([link](#)). GAE is nearly always fatal.

GAE is often not diagnosed until late in the infection. Lesions caused by the infection can be detected using CT or MRI. The live amoebae can be

directly detected in CSF or tissue biopsies. Serological tests are available but generally are not necessary to make a correct diagnosis, since the presence of the organism in CSF is definitive. Some antifungal drugs, like fluconazole, have been used to treat acanthamoebal infections. In addition, a combination of miltefosine and voriconazole (an inhibitor of ergosterol biosynthesis) has recently been used to successfully treat GAE. Even with treatment, however, the mortality rate for patients with these infections is high.



(a)



(b)

(a) Brain tissue from a patient who died of granulomatous amebic encephalitis (GAE) caused by *Balamuthia mandrillaris*. (b) A close-up of the necrosis in the center of the brain section. (credit a, b: modifications of work by the Centers for Disease Control and Prevention)

Note:

- How is granulomatous amoebic encephalitis diagnosed?

Human African Trypanosomiasis

Human African trypanosomiasis (also known as **African sleeping sickness**) is a serious disease endemic to two distinct regions in sub-Saharan Africa. It is caused by the insect-borne hemoflagellate *Trypanosoma brucei*. The subspecies *Trypanosoma brucei rhodesiense* causes **East African trypanosomiasis** (EAT), and another subspecies, *Trypanosoma brucei gambiense* causes **West African trypanosomiasis** (WAT). A few hundred cases of EAT are currently reported each year. [\[footnote\]](#) WAT is more commonly reported and tends to be a more chronic disease. Around 7000 to 10,000 new cases of WAT are identified each year. [\[footnote\]](#)

US Centers for Disease Control and Prevention, “Parasites – African Trypanosomiasis (also known as Sleeping Sickness), East African Trypanosomiasis FAQs,” 2012. Accessed June 30, 2016.

http://www.cdc.gov/parasites/sleepingsickness/gen_info/faqs-east.html.

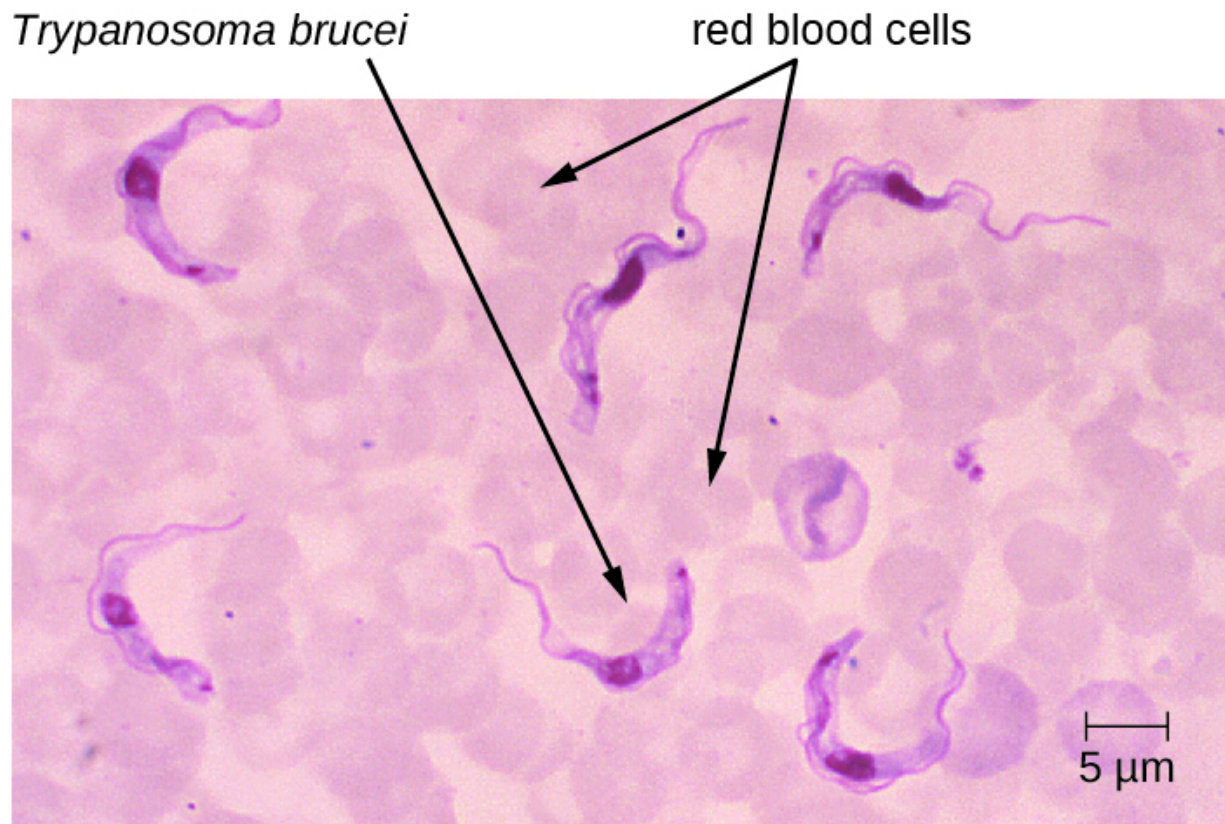
US Centers for Disease Control and Prevention, “Parasites – African Trypanosomiasis (also known as Sleeping Sickness), Epidemiology & Risk Factors,” 2012. Accessed June 30, 2016.

<http://www.cdc.gov/parasites/sleepingsickness/epi.html>.

T. brucei is primarily transmitted to humans by the bite of the tsetse fly (*Glossina* spp.). Soon after the bite of a tsetse fly, a chancre forms at the site of infection. The flagellates then spread, moving into the circulatory system ([\[link\]](#)). These systemic infections result in an undulating fever, during which symptoms persist for two or three days with remissions of about a week between bouts. As the disease enters its final phase, the pathogens move from the lymphatics into the CNS. Neurological symptoms include daytime sleepiness, insomnia, and mental deterioration. In EAT, the disease runs its course over a span of weeks to months. In contrast, WAT often occurs over a span of months to years.

Although a strong immune response is mounted against the trypanosome, it is not sufficient to eliminate the pathogen. Through antigenic variation, *Trypanosoma* can change their surface proteins into over 100 serological types. This variation leads to the undulating form of the initial disease. The initial septicemia caused by the infection leads to high fevers. As the immune system responds to the infection, the number of organisms decrease, and the clinical symptoms abate. However, a subpopulation of the pathogen then alters its surface coat antigens by antigenic variation and evades the immune response. These flagellates rapidly proliferate and cause another bout of disease. If untreated, these infections are usually fatal.

Clinical symptoms can be used to recognize the early signs of African trypanosomiasis. These include the formation of a chancre at the site of infection and **Winterbottom's sign**. Winterbottom's sign refers to the enlargement of lymph nodes on the back of the neck—often indicative of cerebral infections. *Trypanosoma* can be directly observed in stained samples including blood, lymph, CSF, and skin biopsies of chancres from patients. Antibodies against the parasite are found in most patients with acute or chronic disease. Serologic testing is generally not used for diagnosis, however, since the microscopic detection of the parasite is sufficient. Early diagnosis is important for treatment. Before the nervous system is involved, drugs like pentamidine (an inhibitor of nuclear metabolism) and suramin (mechanism unclear) can be used. These drugs have fewer side effects than the drugs needed to treat the second stage of the disease. Once the sleeping sickness phase has begun, harsher drugs including melarsoprol (an arsenic derivative) and eflornithine can be effective. Following successful treatment, patients still need to have follow-up examinations of their CSF for two years to detect possible relapses of the disease. The most effective means of preventing these diseases is to control the insect vector populations.



Trypanosoma brucei, the causative agent of African sleeping sickness, in a human blood smear. (credit: modification of work by the Centers for Disease Control and Prevention)

Note:

- What is the symptom of a systemic *Trypanosoma* infection?
- What are the symptoms of a neurological *Trypanosoma* infection?
- Why are trypanosome infections so difficult to eradicate?

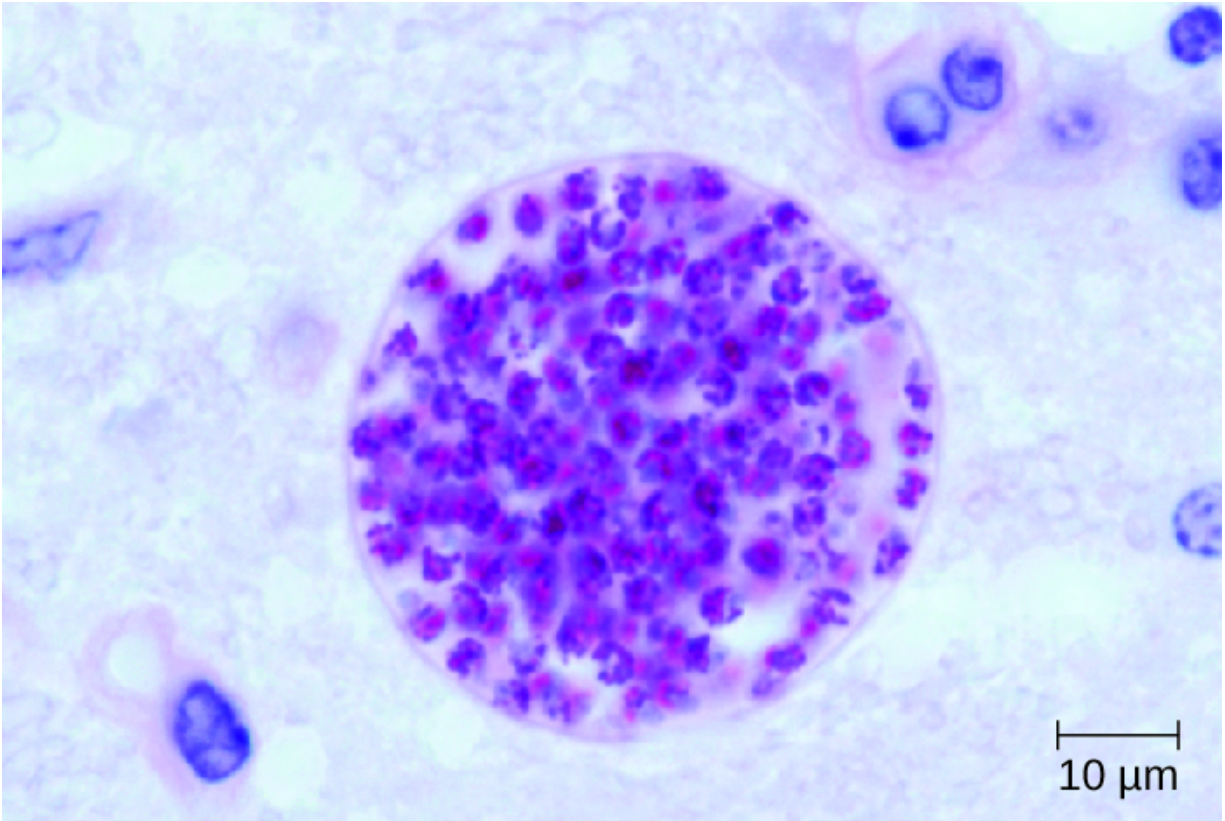
Neurotoxoplasmosis

Toxoplasma gondii is an ubiquitous intracellular parasite that can cause neonatal infections. Cats are the definitive host, and humans can become infected after eating infected meat or, more commonly, by ingesting oocysts shed in the feces of cats (see [Parasitic Infections of the Circulatory and Lymphatic Systems](#)). *T. gondii* enters the circulatory system by passing between the endothelial cells of blood vessels.^[footnote] Most cases of toxoplasmosis are asymptomatic. However, in immunocompromised patients, **neurotoxoplasmosis** caused by *T. gondii* infections are one of the most common causes of brain abscesses.^[footnote] The organism is able to cross the blood-brain barrier by infecting the endothelial cells of capillaries in the brain. The parasite reproduces within these cells, a step that appears to be necessary for entry to the brain, and then causes the endothelial cell to lyse, releasing the progeny into brain tissues. This mechanism is quite different than the method it uses to enter the bloodstream in the first place.^[footnote]

Carruthers, Vern B., and Yasuhiro Suzuki, “Effects of *Toxoplasma gondii* Infection on the Brain,” *Schizophrenia Bulletin* 33, no. 3 (2007): 745-51.
Uppal, Gulshan, “CNS Toxoplasmosis in HIV,” 2015. Accessed June 30, 2016. <http://emedicine.medscape.com/article/1167298-overview#a3>.
Konradt, Christoph, Norikiyo Ueno, David A. Christian, Jonathan H. Delong, Gretchen Harms Pritchard, Jasmin Herz, David J. Bzik et al., “Endothelial Cells Are a Replicative Niche for Entry of *Toxoplasma gondii* to the Central Nervous System,” *Nature Microbiology* 1 (2016): 16001.

The brain lesions associated with neurotoxoplasmosis can be detected radiographically using MRI or CAT scans ([\[link\]](#)). Diagnosis can be confirmed by direct observation of the organism in CSF. RT-PCR assays can also be used to detect *T. gondii* through genetic markers.

Treatment of neurotoxoplasmosis caused by *T. gondii* infections requires six weeks of multi-drug therapy with pyrimethamine, sulfadiazine, and folinic acid. Long-term maintenance doses are often required to prevent recurrence.



This *Toxoplasma gondii* cyst, observed in mouse brain tissue, contains thousands of inactive parasites. (credit: modification of work by USDA)

Note:

- Under what conditions is *Toxoplasma* infection serious?
- How does *Toxoplasma* circumvent the blood-brain barrier?

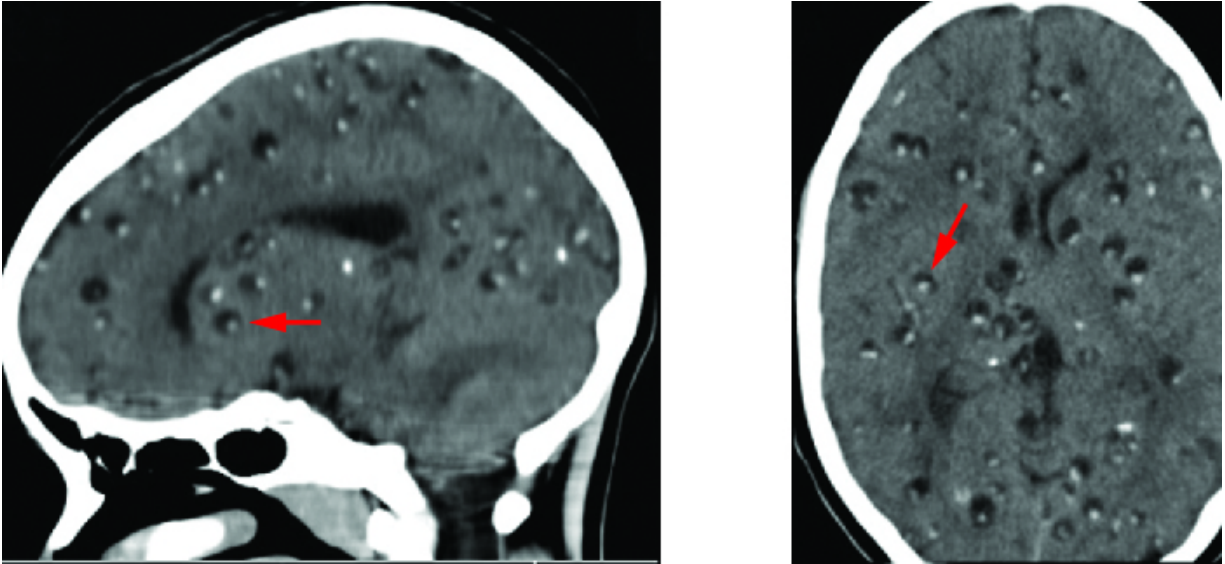
Neurocysticercosis

Cysticercosis is a parasitic infection caused by the larval form of the pork tapeworm, *Taenia solium*. When the larvae invade the brain and spinal cord, the condition is referred to as **neurocysticercosis**. This condition affects millions of people worldwide and is the leading cause of adult onset epilepsy in the developing world.[\[footnote\]](#)

DeGiorgio, Christopher M., Marco T. Medina, Reyna Durón, Chi Zee, and Susan Pietsch Escueta, “Neurocysticercosis,” *Epilepsy Currents* 4, no. 3 (2004): 107-11.

The life cycle of *T. solium* is discussed in [Helminthic Infections of the Gastrointestinal Tract](#). Following ingestion, the eggs hatch in the intestine to form larvae called **cysticerci**. Adult tapeworms form in the small intestine and produce eggs that are shed in the feces. These eggs can infect other individuals through fecal contamination of food or other surfaces. Eggs can also hatch within the intestine of the original patient and lead to an ongoing autoinfection. The cystercerci, can migrate to the blood and invade many tissues in the body, including the CNS.

Neurocysticercosis is usually diagnosed through noninvasive techniques. Epidemiological information can be used as an initial screen; cysticercosis is endemic in Central and South America, Africa, and Asia. Radiological imaging (MRI and CT scans) is the primary method used to diagnose neurocysticercosis; imaging can be used to detect the one- to two-centimeter cysts that form around the parasites ([\[link\]](#)). Elevated levels of eosinophils in the blood can also indicate a parasitic infection. EIA and ELISA are also used to detect antigens associated with the pathogen.



Brain CT scans of sagittal (left) and axial (right) sections of a brain with neurocysticercosis. Numerous cysts are visible in both images, as indicated by the arrows. (credit: modification of work by Segamwenge IL, Kioko NP)

The treatment for neurocysticercosis depends on the location, number, size, and stage of cysticerci present. Anthelmintic chemotherapy includes albendazole and praziquantel. Because these drugs kill viable cysts, they may acutely increase symptoms by provoking an inflammatory response caused by the release of *Taenia* cysticerci antigens, as the cysts are destroyed by the drugs. To alleviate this response, corticosteroids that cross the blood-brain barrier (e.g., dexamethasone) can be used to mitigate these effects. Surgical intervention may be required to remove intraventricular cysts.

Note:

Parasitic Diseases of the Nervous System

Parasites that successfully invade the nervous system can cause a wide range of neurological signs and symptoms. Often, they inflict lesions that can be visualized through radiologic imaging. A number of these infections

are fatal, but some can be treated (with varying levels of success) by antimicrobial drugs ([\[link\]](#)).

Parasitic Diseases of the Nervous System					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Granulomatous amoebic encephalitis (GAE)	<i>Acanthamoeba</i> spp., <i>Balamuthia mandrillaris</i>	Inflammation, lesions in CNS, almost always fatal	Freshwater amoebae invade CNS via breaks in skin or sinuses	CT scan, MRI, CSF	Fluconazole, miltefosine, voriconazole
Human African trypanosomiasis	<i>Trypanosoma brucei</i> <i>gambiense</i> , <i>T. brucei rhodesiense</i>	Chancre, Winterbottom's sign, undulating fever, lethargy, insomnia, usually fatal if untreated	Protozoan transmitted via bite of tsetse fly	Blood smear	Pentamidine and suramine (initial phase); melarsoprol and eflornithine (final phase)
Neurocysticercosis	<i>Taenia solium</i>	Brain cysts, epilepsy	Ingestion of tapeworm eggs in fecally contaminated food or surfaces	CT scan, MRI	Albendazole, praziquantel, dexamethasone
Neurotoxoplasmosis	<i>Toxoplasma gondii</i>	Brain abscesses, chronic encephalitis	Protozoan transmitted via contact with oocytes in cat feces	CT scan, MRI, CSF	Pyrimethamine, sulfadiazine, folinic acid
Primary amoebic meningoencephalitis (PAM)	<i>Naegleria fowleri</i>	Headache, seizures, coma, almost always fatal	Freshwater amoebae invade brain via nasal passages	CSF, IFA, PCR	Miltefosine (experimental)

Note:

- What neurological condition is associated with neurocysticercosis?
- How is neurocysticercosis diagnosed?

Key Concepts and Summary

- **Neuromycoses** are uncommon in immunocompetent people, but immunocompromised individuals with fungal infections have high mortality rates. Treatment of neuromycoses require prolonged therapy with antifungal drugs at low doses to avoid side effects and overcome the effect of the blood-brain barrier.
- Some protist infections of the nervous systems are fatal if not treated, including **primary amoebic meningitis**, **granulomatous amoebic encephalitis**, **human African trypanosomiasis**, and **neurotoxoplasmosis**.
- The various forms of amoebic encephalitis caused by the different amoebic infections are typically fatal even with treatment, but they are rare.
- **African trypanosomiasis** is a serious but treatable disease endemic to two distinct regions in sub-Saharan Africa caused by the insect-borne hemoflagellate *Trypanosoma brucei*.
- **Neurocysticercosis** is treated using antihelminthic drugs or surgery to remove the large cysts from the CNS.

Multiple Choice

Exercise:

Problem:

Which of these diseases results in meningitis caused by an encapsulated yeast?

- a. cryptococcosis
- b. histoplasmosis
- c. candidiasis
- d. coccidiomycosis

Solution:

A

Exercise:

Problem:

What kind of stain is most commonly used to visualize the capsule of cryptococcus?

- a. Gram stain
- b. simple stain
- c. negative stain
- d. fluorescent stain

Solution:

C

Exercise:**Problem:**

Which of the following is the causative agent of East African trypanosomiasis?

- a. *Trypanosoma cruzi*
- b. *Trypanosoma vivax*
- c. *Trypanosoma brucei rhodanese*
- d. *Trypanosoma brucei gambiense*

Solution:

C

Exercise:**Problem:**

Which of the following is the causative agent of primary amoebic meningoencephalitis?

- a. *Naegleria fowleri*

- b. *Entameba histolyticum*
- c. *Amoeba proteus*
- d. *Acanthamoeba polyphaga*

Solution:

A

Exercise:

Problem: What is the biological vector for African sleeping sickness?

- a. mosquito
- b. tsetse fly
- c. deer tick
- d. sand fly

Solution:

B

Exercise:

Problem: How do humans usually contract neurocysticercosis?

- a. the bite of an infected arthropod
- b. exposure to contaminated cat feces
- c. swimming in contaminated water
- d. ingestion of undercooked pork

Solution:

D

Exercise:

Problem:

Which of these is the most important cause of adult onset epilepsy?

- a. neurocysticercosis
 - b. neurotoxoplasmosis
 - c. primary amoebic meningoencephalitis
 - d. African trypanosomiasis
-

Solution:

A

Fill in the Blank

Exercise:

Problem:

The _____ is the main virulence factor of *Cryptococcus neoformans*.

Solution:

capsule

Exercise:

Problem:

The drug of choice for fungal infections of the nervous system is _____.

Solution:

Amphotericin B

Exercise:

Problem: The larval forms of a tapeworm are known as _____.

Solution:

cysticerci

Exercise:

Problem:

_____ sign appears as swollen lymph nodes at the back of the neck in early African trypanosomiasis.

Solution:

Winterbottom's

Exercise:

Problem:

_____ African trypanosomiasis causes a chronic form of sleeping sickness.

Solution:

West

Exercise:

Problem: The definitive host for *Toxoplasma gondii* is _____.

Solution:

cats

Exercise:

Problem:

Trypanosomes can evade the immune response through _____ variation.

Solution:

antigenic

Short Answer**Exercise:****Problem:**

Why do nervous system infections by fungi require such long treatment times?

Exercise:**Problem:**

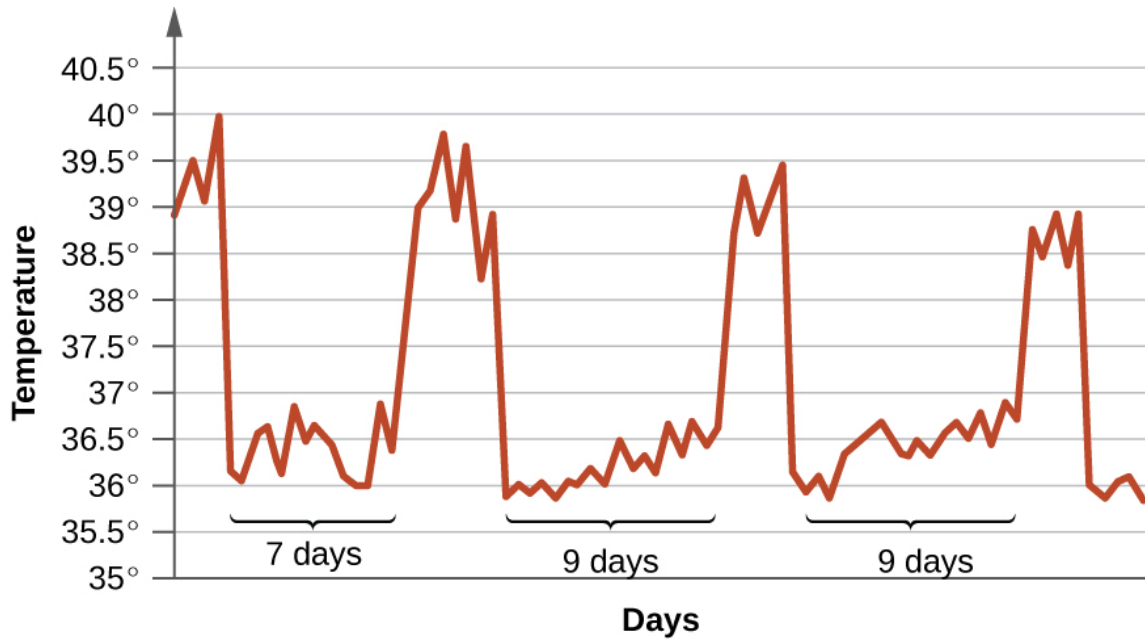
Briefly describe how humans are infected by *Naegleria fowleri*.

Exercise:**Problem:**

Briefly describe how humans can develop neurocysticercosis.

Critical Thinking**Exercise:****Problem:**

The graph shown tracks the body temperature of a patient infected with *Trypanosoma brucei*. How would you describe this pattern, and why does it occur?



(credit: modification of work by Wellcome Images)

Exercise:

Problem:

Fungal meningoencephalitis is often the ultimate cause of death for AIDS patients. What factors make these infections more problematic than those of bacterial origin?

Exercise:

Problem:

Compare East African trypanosomiasis with West African trypanosomiasis.

Glossary

- **454 sequencing (pyrosequencing)** a next generation sequencing technique in which fragmented DNA has DNA adapters attached, is amplified by PCR, is attached to a bead, and then placed into a well with sequencing reagents, and the flash of light produced by the release of pyrophosphate on addition of a nucleotide is monitored
- **5' cap** methylguanosine nucleotide added to 5' end of a eukaryotic primary transcript
- **70S ribosome** a ribosome composed of 50S and 30S subunits
- **80S ribosome** cytoplasmic eukaryotic ribosome composed of 60S and 40S subunits

A

- **α -helix** secondary structure consisting of a helix stabilized by hydrogen bonds between nearby amino acid residues in a polypeptide
- **A (aminoacyl) site** functional site of an intact ribosome that binds incoming charged aminoacyl tRNAs
- **A-B exotoxin** class of exotoxin that contains A subunits, which enter the cell and disrupt cellular activities, and B subunits, which bind to host cell receptors
- **ABO blood group system** set of glycoprotein antigens found on the surface of red blood cells; the presence or absence of specific carbohydrates determining blood type
- **absorbance** when a molecule captures energy from a photon and vibrates or stretches, using the energy
- ***Acanthamoeba keratitis*** a condition characterized by damage to the cornea and possible blindness caused by parasitic infection of the protozoan *Acanthamoeba*
- **acellular** not made of cells
- **acid-fast stain** a stain that differentiates cells that have waxy mycolic acids in their gram-positive cell walls

- **acidic dye** a chromophore with a negative charge that attaches to positively charged structures
- **acidophile** organism that grows optimally at a pH near 3.0
- **acne** a skin disease in which hair follicles or pores become clogged, leading to the formation of comedones and infected lesions
- **acquired immunodeficiency syndrome (AIDS)** disease caused by HIV, characterized by opportunistic infections and rare cancers
- **actin** a protein that polymerizes to form microfilaments
- **activation energy** energy needed to form or break chemical bonds and convert a reactant or reactants to a product or products
- **activator** protein that increases the transcription of a gene in response to an external stimulus
- **active carrier** an infected individual who can transmit the pathogen to others regardless of whether symptoms are currently present
- **active immunity** stimulation of one's own adaptive immune responses
- **active site** location within an enzyme where substrate(s) bind
- **acute disease** disease of a relatively short duration that develops and progresses in a predictable pattern
- **acute glomerulonephritis** inflammation of the glomeruli of the kidney, probably resulting from deposition of immune complexes and an autoimmune response caused by self-antigen mimicry by a pathogen
- **acute necrotizing ulcerative gingivitis** a severe form of gingivitis, also called trench mouth
- **acute otitis media** inflammatory disease of the middle ear resulting from a microbial infection
- **acute rheumatic fever** sequela of streptococcal pharyngitis; comorbidities include arthritis and carditis
- **acute-phase proteins** antimicrobial molecules produced by liver cells in response to pathogen-induced stimulation events
- **acyclovir** antiviral guanosine analog; inhibits DNA replication
- **adaptive immunity** third-line defense characterized by specificity and memory
- **Addison disease** autoimmune disease affecting adrenal gland function
- **adenine** purine nitrogenous base found in nucleotides
- **adenosine diphosphate (ADP)** nucleotide derivative and relative of ATP containing only one high-energy phosphate bond

- **adenosine monophosphate (AMP)** adenine molecule bonded to a ribose molecule and to a single phosphate group, having no high-energy phosphate bonds
- **adenosine triphosphate (ATP)** energy currency of the cell; a nucleotide derivative that safely stores chemical energy in its two high-energy phosphate bonds
- **adhesins** molecules on the surface of pathogens that promote colonization of host tissue
- **adhesion** the capability of microbes to attach to host cells
- **aerobic respiration** use of an oxygen molecule as the final electron acceptor of the electron transport system
- **aerotolerant anaerobe** organism that does not use oxygen but tolerates its presence
- **affinity maturation** function of the immune system by which B cells, upon re-exposure to antigen, are selected to produce higher affinity antibodies
- **affinity** measure of how tightly an antibody-binding site binds to its epitope
- **aflatoxin** chemical produced by the fungus *Aspergillus flavus*; both a toxin and the most potent known natural carcinogen
- **African sleeping sickness** see *human African trypanosomiasis*
- **agarose gel electrophoresis** a method for separating populations of DNA molecules of varying sizes by differential migration rates caused by a voltage gradient through a horizontal gel matrix
- **agglutination** binding of different pathogen cells by Fab regions of the same antibody to aggregate and enhance elimination from body
- **agranulocytes** leukocytes that lack granules in the cytoplasm
- **alarmone** small intracellular derivative of a nucleotide that signals a global bacterial response (i.e., activating a regulon of operons) to an environmental stress
- **albendazole** antihelminthic drug of the benzimidazole class that binds to helminthic β -tubulin, preventing microtubule formation
- **algae** (singular: alga) any of various unicellular and multicellular photosynthetic eukaryotic organisms; distinguished from plants by their lack of vascular tissues and organs
- **alkaliphile** organism that grows optimally at pH above 9.0

- **alkylating agent** type of strong disinfecting chemical that acts by replacing a hydrogen atom within a molecule with an alkyl group, thereby inactivating enzymes and nucleic acids
- **allergen** antigen capable of inducing type I hypersensitivity reaction
- **allergy** hypersensitivity response to an allergen
- **allograft** transplanted tissue from an individual of the same species that is genetically different from the recipient
- **allosteric activator** molecule that binds to an enzyme's allosteric site, increasing the affinity of the enzyme's active site for the substrate(s)
- **allosteric site** location within an enzyme, other than the active site, to which molecules can bind, regulating enzyme activity
- **allylamines** class of antifungal drugs that inhibit ergosterol biosynthesis at an early point in the pathway
- **Alphaproteobacteria** class of Proteobacteria that are all oligotrophs
- **alveoli** cul-de-sacs or small air pockets within the lung that facilitate gas exchange
- **amantadine** antiviral drug that targets the influenza virus by preventing viral escape from endosomes upon host cell uptake, thus preventing viral RNA release and subsequent viral replication
- **amensalism** type of symbiosis in which one population harms the other but remains unaffected itself
- **Ames test** method that uses auxotrophic bacteria to detect mutations resulting from exposure to potentially mutagenic chemical compounds
- **amino acid** a molecule consisting of a hydrogen atom, a carboxyl group, and an amine group bonded to the same carbon. The group bonded to the carbon varies and is represented by an *R* in the structural formula
- **aminoacyl-tRNA synthetase** enzyme that binds to a tRNA molecule and catalyzes the addition of the correct amino acid to the tRNA
- **aminoglycosides** protein synthesis inhibitors that bind to the 30S subunit and interfere with the ribosome's proofreading ability, leading to the generation of faulty proteins that insert into and disrupt the bacterial cytoplasmic membrane
- **amoebiasis** intestinal infection caused by *Entamoeba histolytica*
- **amoebic dysentery** severe form of intestinal infection caused by *Entamoeba histolytica*, characterized by severe diarrhea with blood and mucus

- **amphipathic** a molecule containing both polar and nonpolar parts
- **amphitrichous** having two flagella or tufts of multiple flagella, with one flagellum or tuft located at each end of the bacterial cell
- **amphotericin B** antifungal drug of the polyene class that is used to treat several systemic fungal infections
- **amplitude** the height of a wave
- **anabolism** chemical reactions that convert simpler molecules into more complex ones
- **anaerobe chamber** closed compartment used to handle and grow obligate anaerobic cultures
- **anaerobe jar** container devoid of oxygen used to grow obligate anaerobes
- **anaerobic respiration** use of a non-oxygen inorganic molecule, like CO₂, nitrate, nitrite, oxidized iron, or sulfate, as the final electron acceptor at the end of the electron transport system
- **analytical epidemiology** study of disease outbreaks to establish associations between an agent and a disease state through observational studies comparing groups of individuals
- **anaphylactic shock** another term for anaphylaxis
- **anaphylaxis** systemic and potentially life-threatening type I hypersensitivity reaction
- **anergy** peripheral tolerance mechanism that prevents self-reactive T cells from being activated by self-antigens through lack of co-stimulation
- **annealing** formation of hydrogen bonds between the nucleotide base pairs of two single-stranded complementary nucleic acid sequences
- **anoxygenic photosynthesis** type of photosynthesis found in many photosynthetic bacteria, including the purple and green bacteria, where an electron donor other than H₂O is used to replace an electron lost by a reaction center pigment, resulting no oxygen production
- **anthrax** a disease caused by *Bacillus anthracis*; the cutaneous form causes a skin lesion to develop; gastrointestinal and inhalation anthrax have high mortality rates
- **antibiogram** compilation of the antimicrobial susceptibilities recorded for local bacterial strains, which is useful for monitoring local trends in antimicrobial resistance and aiding the prescription of appropriate empiric antibacterial therapy

- **antibiotic-associated diarrhea** diarrhea that develops after antibiotic treatment as a result of disruption to the normal microbiota; *C. difficile* is a particularly serious example
- **antibody screen** test to make sure that a potential blood recipient has not produced antibodies to antigens other than the ABO and Rh antigens
- **antibody** Y-shaped glycoprotein molecule produced by B cells that binds to specific epitopes on an antigen
- **antibody-dependent cell-mediated cytotoxicity (ADCC)** mechanism by which large pathogens are marked for destruction by specific antibodies and then killed by secretion of cytotoxins by natural killer cells, macrophages, or eosinophils
- **anticodon** three-nucleotide sequence of a mature tRNA that interacts with an mRNA codon through complementary base pairing
- **antigen (also, immunogen)** a molecule that stimulates an adaptive immune response
- **antigenic** able to stimulate an adaptive immune response
- **antigenic drift** form of slight antigenic variation that occurs because of point mutations in the genes that encode surface proteins
- **antigenic shift** form of major antigenic variation that occurs because of gene reassortment
- **antigenic variation** changing of surface antigens (carbohydrates or proteins) such that they are no longer recognized by the host's immune system
- **antigen-presenting cells (APC)** macrophages, dendritic cells, and B cells that process and present foreign pathogen antigens for the purpose of activating T cells and adaptive immune defenses
- **antimetabolites** compounds that are competitive inhibitors for bacterial metabolic enzymes
- **antimicrobial drugs** chemical compounds, including naturally produced drugs, semisynthetic derivatives, and synthetic compounds, that target specific microbial structures and enzymes, killing specific microbes or inhibiting their growth
- **antimicrobial peptides (AMPs)** class of nonspecific, cell-derived chemical mediators with broad-spectrum antimicrobial properties
- **antiparallel** two strands of DNA helix oriented in opposite directions; one strand is oriented in the 5' to 3' direction, while the other is

oriented in the 3' to 5' direction

- **antisense RNA** small noncoding RNA molecules that inhibit gene expression by binding to mRNA transcripts via complementary base pairing
- **antisense strand** transcription template strand of DNA; the strand that is transcribed for gene expression
- **antiseptis** protocol that removes potential pathogens from living tissue
- **antiseptic** antimicrobial chemical that can be used safely on living tissue
- **antiserum** serum obtained from an animal containing antibodies against a particular antigen that was artificially introduced to the animal
- **apoenzyme** enzyme without its cofactor or coenzyme
- **apoptosis** programmed and organized cell death without lysis of the cell
- **arachnoid mater** middle membrane surrounding the brain that produces cerebrospinal fluid
- **arboviral encephalitis** infection by an arthropod-borne virus that results in an inflammation of the brain
- **arbovirus** any of a variety of viruses that are transmitted by arthropod vectors
- **archaea** any of various unicellular prokaryotic microorganisms, typically having cell walls containing pseudopeptidoglycan
- **Archaea** domain of life separate from the domains Bacteria and Eukarya
- **artemisinin** antiprotozoan and antifungal drug effective against malaria that is thought to increase intracellular levels of reactive oxygen species in target microbes
- **artery** large, thick-walled vessel that carries blood from the heart to the body tissues
- **Arthus reaction** localized type III hypersensitivity
- **artificial active immunity** immunity acquired through exposure to pathogens and pathogen antigens through a method other than natural infection
- **artificial passive immunity** transfer of antibodies produced by a donor to another individual for the purpose of preventing or treating disease

- **ascariasis** soil-transmitted intestinal infection caused by the large nematode roundworm *Ascaris lumbricoides*
- **ascocarps** cup-shaped fruiting bodies of an ascomycete fungus
- **ascospore** asexual spore produced by ascomycete fungi
- **ascus** structure of ascomycete fungi containing spores
- **asepsis** sterile state resulting from proper use of microbial control protocols
- **aseptic technique** method or protocol designed to prevent microbial contamination of sterile objects, locations, or tissues
- **aspergillosis** fungal infection caused by the mold *Aspergillus*; immunocompromised patients are primarily at risk
- **asymptomatic carrier** an infected individual who exhibits no signs or symptoms of disease yet is capable of transmitting the pathogen to others
- **asymptomatic** not exhibiting any symptoms of disease
- **atomic force microscope** a scanning probe microscope that uses a thin probe that is passed just above the specimen to measure forces between the atoms and the probe
- **ATP synthase** integral membrane protein that harnesses the energy of the proton motive force by allowing hydrogen ions to diffuse down their electrochemical gradient, causing components of this protein to spin, making ATP from ADP and P_i
- **attachment** binding of phage or virus to host cell receptors
- **attenuation** regulatory system of prokaryotes whereby secondary stem-loop structures formed within the 5' end of an mRNA being transcribed determine both if transcription to complete the synthesis of this mRNA will occur and if this mRNA will be used for translation
- **autoclave** specialized device for the moist-heat sterilization of materials through the application of pressure to steam, allowing the steam to reach temperatures above the boiling point of water
- **autocrine function** refers to a cytokine signal released from a cell to a receptor on its own surface
- **autograft** tissue transplanted from a location on an individual to a different location on the same individual
- **autoimmune disease** loss of tolerance to self, resulting in immune-mediated destruction of self cells and tissues

- **autoinducer** signaling molecule produced by a bacterial cell that can modify the activity of surrounding cells; associated with quorum sensing
- **autoradiography** the method of producing a photographic image from radioactive decay; in molecular genetics the method allows the visualization of radioactively-labeled DNA probes that have hybridized to a nucleic acid sample
- **autotroph** organism that converts inorganic carbon dioxide into organic carbon
- **auxotroph** nutritional mutant with a loss-of-function mutation in a gene encoding the biosynthesis of a specific nutrient such as an amino acid
- **avidity** strength of the sum of the interactions between an antibody and antigen
- **axon** long projection of a neuron along which an electrochemical signal is transmitted
- **azithromycin** semisynthetic macrolide with increased spectrum of activity, decreased toxicity, and increased half-life compared with erythromycin

B

- **β -lactamases** bacterially produced enzymes that cleave the β -lactam ring of susceptible β -lactam antimicrobials, rendering them inactive and conferring resistance
- **β -lactams** group of antimicrobials that inhibit cell wall synthesis; includes the penicillins, cephalosporins, carbapenems, and monobactams; inhibits the transpeptidase cross-linking activity of penicillin-binding proteins
- **β -oxidation** process of fatty acid degradation that sequentially removes two-carbon acetyl groups, producing NADH and FADH₂, on entry into the Krebs cycle
- **β -pleated sheet** secondary structure consisting of pleats formed by hydrogen bonds between localized segments of amino acid residues on the backbone of the polypeptide chain

- **B-cell receptors (BCRs)** membrane-bound IgD and IgM antibody that bind specific antigen epitopes with Fab antigen-binding region
- **B lymphocyte** antibody-producing cells of humoral immunity; B cell
- **babesiosis** tickborne protozoan infection caused by *Babesia* spp. and characterized by malaise, fatigue, fever, headache, myalgia, and joint pain
- **bacillary dysentery** gastrointestinal illness caused by *Shigella* bacteria, also called shigellosis
- **bacillus** (bacilli) rod-shaped prokaryotic cell
- **bacitracin** group of structurally similar peptides that block the movement of peptidoglycan precursors across the cell membrane, inhibiting peptidoglycan synthesis
- **bacteremia** condition marked by the presence of bacteria in the blood
- **bacteria** (singular: bacterium) any of various unicellular prokaryotic microorganisms typically (but not always) having cell walls that contain peptidoglycan
- **bacterial lawn** layer of confluent bacterial growth on an agar plate
- **bacterial meningitis** bacterial infection that results in an inflammation of the meninges
- **bacterial vaginosis** a condition caused by an overgrowth of bacteria in the vagina that may or may not cause symptoms
- **bactericidal** irreversible inhibition of a microbe's ability to divide
- **bactericide** chemical or physical treatment that kills bacteria
- **bacteriochlorophylls** green, purple, or blue pigments of bacteria; they are similar to chlorophyll of plants
- **bacteriology** the study of bacteria
- **bacteriophage** virus that infects bacteria
- **bacteriostatic** having the ability to inhibit bacterial growth, generally by means of chemical or physical treatment; reversible inhibition of a microbe's ability to divide
- **barophile** organism that grows under high atmospheric pressure
- **basal body** component of eukaryotic flagellum or cilium composed of nine microtubule triplets and attaches the flagellum or cilium to the cell
- **base sequence** identity of the specific nucleotides present in a nucleic acid strand and their order within the strand

- **basic dye** a chromophore with a positive charge that attaches to negatively charged structures
- **basidia (basidium, sing.)** small club-shaped structures of basidiomycete fungi where basidiospores are produced
- **basidiocarps** fruiting bodies of basidiomycete fungi
- **basidiospores** spores produced sexually via budding in basidiomycete fungi
- **basophils** leukocytes with granules containing histamine and other chemicals that facilitate allergic responses and inflammation when released
- **benzimidazoles** class of antihelminthic drugs that bind to helminthic β -tubulin, preventing microtubule formation
- **Betaproteobacteria** class of Proteobacteria that are all eutrophs
- **binary fission** predominant form of bacterial reproduction in which one cell divides into two daughter cells of equal size, which separate, each offspring receiving a complete copy of the parental genome
- **binocular** having two eyepieces
- **binomial nomenclature** a universal convention for the scientific naming of organisms using Latinized names for genus and species
- **biofilm** complex ecosystem of bacteria embedded in a matrix
- **biogeochemical cycle** recycling of inorganic matter between living organisms and their nonliving environment
- **bioinformatics** the analysis of large amounts of information required for interpretation of these data
- **biological transmission** movement of a pathogen between hosts facilitated by a biological vector in which the pathogen grows and reproduces
- **biological vector** an animal (typically an arthropod) that is infected with a pathogen and is capable of transmitting the pathogen from one host to another
- **biomarker** a protein expressed by a cell or tissue that is indicative of disease
- **biomolecule** a molecule that is part of living matter
- **bioremediation** use of microbes to remove xenobiotics or environmental pollutants from a contaminated site
- **biosynthesis** replication of viral genome and other protein components

- **biotechnology** the science of using living systems to benefit humankind
- **bisbiguanide** type of chemical compound with antiseptic properties; disrupts cell membranes at low concentrations and causes congealing of intracellular contents at high concentrations
- **blastomycosis** fungal disease associated with infections by *Blastomyces dermatitidis*; can cause disfiguring scarring of the hands and other extremities
- **blepharitis** inflammation of the eyelids
- **blocking antibodies** antigen-specific antibodies (usually of the IgG type) produced via desensitization therapy
- **blood-brain barrier** tight cell junctions of the endothelia lining the blood vessels that serve the central nervous system, preventing passage of microbes from the bloodstream into the brain and cerebrospinal fluid
- **blue-white screening** a technique commonly used for identifying transformed bacterial cells containing recombinant plasmids using *lacZ*-encoding plasmid vectors
- **blunt ends** ends of DNA molecules lacking single-stranded complementary overhangs that are produced when some restriction enzymes cut DNA
- **botulism** form of flaccid paralysis caused by the ingestion of a neurotoxin produced by *Clostridium botulinum*
- **bradykinin** activated form of a proinflammatory molecule induced in the presence of invader microbes; opens gaps between cells in blood vessels, allowing fluid and cells to leak into surrounding tissue
- **bridge reaction** reaction linking glycolysis to the Krebs cycle during which each pyruvate is decarboxylated and oxidized (forming NADH), and the resulting two-carbon acetyl group is attached to a large carrier called coenzyme A, resulting in the formation of acetyl-CoA and CO₂; also called the *transition reaction*
- **brightfield microscope** a compound light microscope with two lenses; it produces a dark image on a bright background
- **broad-spectrum antimicrobial** drug that targets many different types of microbes
- **bronchi** major air passages leading to the lungs after bifurcating at the windpipe

- **bronchioles** smaller air passages within the lung that are formed as the bronchi become further subdivided
- **bronchitis** inflammation of the bronchi
- **brucellosis** zoonotic disease caused by bacteria of the genus *Brucella* that results in undulant fever
- **bubo** swollen, inflamed lymph node that forms as a result of a microbial infection
- **bubonic plague** most common form of plague in humans, marked by the presence of swollen lymph nodes (buboes)
- **budding** unequal reproductive division in which a smaller cell detaches from the parent cell
- **budding yeasts** yeasts that divide by budding off of daughter cells
- **Burkitt lymphoma** disease characterized by rapidly growing solid tumor; caused by Epstein-Barr virus (HHV-4)
- **burst** release of new virions by a lysed host cell infected by a virus
- **burst size** the number of virions released from a host cell when it is lysed because of a viral infection

C

- **Calvin-Benson cycle** most common CO₂ fixation pathway in most photoautotrophs; involves light-independent reactions of photosynthesis that occur in the cytoplasm of photosynthetic bacteria and in the stroma of eukaryotic chloroplasts
- ***Campylobacter jejuni* gastroenteritis** gastroenteritis caused by *C. jejuni*; generally mild but sometimes with serious complications
- **candidiasis** fungal infection caused by *Candida* spp., especially *C. albicans*; can affect various regions of the body, e.g., skin (cutaneous candidiasis), oral cavity (oral thrush), or vagina (yeast infection)
- **candle jar** container with a tight-fitting lid in which a burning candle consumes oxygen and releases carbon dioxide, thereby creating an environment suitable for capnophiles
- **capillary** small blood vessel found in the interstitial space of tissue; delivers nutrients and oxygen, and removes waste products
- **capnophile** organism that requires carbon dioxide levels higher than atmospheric concentration

- **capsid** protein coat surrounding the genome of the virus
- **capsomere** individual protein subunits that make up the capsid
- **capsule staining** a negative staining technique that stains around a bacterial capsule while leaving the capsule clear
- **capsule** type of glycocalyx with organized layers of polysaccharides that aid in bacterial adherence to surfaces and in evading destruction by immune cells
- **carbapenem-resistant Enterobacteriaceae (CRE)** group of bacteria that have developed resistance to all β -lactams, including carbapenems, and many other drug classes
- **carbohydrate** the most abundant type of biomolecule, consisting of carbon, hydrogen, and oxygen
- **carbon skeleton** chain of carbon atoms to which one or more functional groups are bound
- **carboxysome** an inclusion composed of an outer shell of thousands of protein subunits. Its interior is filled with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase, which are both used for carbon metabolism
- **carbuncle** abscess containing a large, deep, purulent skin lesion
- **carcinogen** agent that causes cancer
- **case-control study** a type of observational study in which a group of affected individuals are compared, usually retrospectively, to a similar group of unaffected individuals
- **catabolic activator protein (CAP)/cAMP receptor protein (CRP)** protein that, when bound to cAMP in the presence of low levels of glucose, binds to the promoters of operons that control the processing of alternative sugars
- **catabolism** chemical reactions that break down complex molecules into simpler ones
- **catabolite repression** repression of the transcription of operons encoding enzymes for the use of substrates other than glucose when glucose levels are high
- **catalase** enzyme that breaks down hydrogen peroxide to water and oxygen
- **catalyst** molecule that increases the rate of a chemical reaction but is not used or changed during the chemical reaction and, thus, is reusable

- **catarrhal stage** in pertussis, a disease stage marked by inflammation of the mucous membranes combined with excessive secretions
- **cat-scratch disease** bacterial infection of the lymph nodes caused by *Bartonella henselae*; frequently transmitted via a cat scratch
- **causative agent** the pathogen or substance responsible for causing a particular disease; etiologic agent
- **CCA amino acid binding end** region of a mature tRNA that binds to an amino acid
- **celiac disease** disease largely of the small intestine caused by an immune response to gluten that results in the production of autoantibodies and an inflammatory response
- **cell envelope** the combination of external cellular structures (e.g., plasma membrane, cell wall, outer membrane, glycocalyxes) that collectively contain the cytoplasm and internal structures of a cell
- **cell membrane** lipid bilayer with embedded proteins and carbohydrates that defines the boundary of the cell (also called the cytoplasmic membrane or plasma membrane)
- **cell morphology** cell shape, structure, and arrangement, as viewed microscopically
- **cell theory** the theory that all organisms are composed of cells and that the cell is the fundamental unit of life
- **cell wall** a structure in the cell envelope of some cells that helps the cell maintain its shape and withstand changes in osmotic pressure
- **cellular immunity** adaptive immunity involving T cells and the destruction of pathogens and infected cells
- **cellulitis** a subcutaneous skin infection that develops in the dermis or hypodermis, resulting in a red, painful inflammation
- **cellulose** a structural polysaccharide composed of glucose monomers linked together in a linear chain by glycosidic bonds
- **Centers for Disease Control and Prevention (CDC)** the national public health agency in the United States
- **central dogma** scientific principle explaining the flow of genetic information from DNA to RNA to protein
- **central nervous system (CNS)** portion of the nervous system made up of the brain and spinal cord
- **central tolerance** negative selection of self-reactive T cells in thymus

- **centriole** a component of a centrosome with the structural array of nine parallel microtubules arranged in triplets; involved in eukaryotic cell division
- **centrosome** a microtubule-organizing center for the mitotic spindle found in animal cells; it separates chromosomes during cell division and is composed of a pair of centrioles positioned at right angles to each other
- **cephalosporins** a group of cell wall synthesis inhibitors within the class of β -lactams
- **cercarial dermatitis** inflammation of the skin caused by a reaction to cercaria of *Schistosoma* spp., which can penetrate the skin and blood vessels; also called swimmer's itch or clam digger's itch
- **cerebrospinal fluid (CSF)** sterile liquid produced in the brain that fills the subarachnoid space of the brain and spinal column
- **cervix** the part of the uterus that connects to the vagina
- **CFB group** phylum consisting of the gram-negative, rod-shaped nonproteobacteria genera *Cytophaga*, *Fusobacterium*, and *Bacteroides*
- **Chagas disease** potentially fatal protozoan infection caused by *Trypanosoma cruzi* and endemic to Central and South America; transmitted by the triatomine bug (kissing bug)
- **chancroid** an STI caused by *Haemophilus ducreyi* that produces soft chancres on genitals
- **charged tRNA** activated tRNA molecule carrying its cognate amino acid
- **chemical mediators** chemicals or enzymes produced by a variety of cells; provide nonspecific antimicrobial defense mechanisms
- **chemically defined media** media in which all components are chemically defined
- **chemiosmosis** flow of hydrogen ions across the membrane through ATP synthase
- **chemokines** chemotactic cytokines that recruit specific subsets of leukocytes to infections, damaged tissue, and sites of inflammation
- **chemotaxis** directional movement of a cell in response to a chemical attractant
- **chemotroph** organism that gets its energy from the transfer of electrons originating from chemical compounds

- **chickenpox** common childhood disease caused by the varicella-zoster virus and marked by the formation of pustular lesions on the trunk
- **chikungunya fever** mosquito-borne viral disease caused by the chikungunya virus and characterized by high fever, joint pain, rash, and blisters
- **chirality** property of stereoisomer molecules by which their structures are nonsuperimposable mirror-images
- **chitin** polysaccharide that is an important component of fungal cell walls
- **chlamydia** a common STI caused by *Chlamydia trachomatis*
- **chloramphenicol** protein synthesis inhibitor with broad-spectrum activity that binds to the 50S subunit, inhibiting peptide bond formation
- **chlorophyll** a type of photosynthetic pigment found in some prokaryotic and eukaryotic cells
- **chloroplast** organelle found in plant and algal cells in which photosynthesis occurs
- **cholera** gastrointestinal illness caused by *Vibrio cholera* characterized by severe diarrhea
- **chromatin** combination of DNA with DNA binding proteins
- **chromogenic substrate** colorless substrate (chromogen) that is converted into a colored end product by the enzyme
- **chromophores** pigments that absorb and reflect particular wavelengths of light (giving them a color)
- **chromosome** discrete DNA structure within a cell that controls cellular activities
- **chronic disease** any disease that progresses and persists over a long time
- **chronic granulomatous disease** primary immunodeficiency caused by an impaired ability of phagocytic cells to kill ingested bacteria in the phagolysosome
- **chronic wasting disease** prion disease of deer and elk in the United States and Canada
- **cilia (singular: cilium)** short filamentous structures found on some eukaryotic cells; each is composed of microtubules in a 9+2 array, and may be used for locomotion, feeding, and/or movement of extracellular particles that come in contact with the cell

- **ciliated epithelial cells** hair-like cells in the respiratory tract that beat, pushing mucus secretions and trapped debris away from the sensitive tissues of the lungs
- **ciliates** protists with cilia (Ciliophora), including *Paramecium* and *Stentor*, classified within the Chromalveolata
- **cisternae** the sacs of the endoplasmic reticulum
- **citric acid cycle** see *Krebs cycle*
- **class switching** genetic rearrangement of constant region gene segments in plasma cells to switch antibody production from IgM to IgG, IgA, or IgE
- **clindamycin** semisynthetic protein synthesis inhibitor of the lincosamide class that binds to the 50S subunit, inhibiting peptide bond formation
- **clone** a genetically identical cell or individual
- ***Clostridium perfringens* gastroenteritis** relatively mild gastrointestinal illness caused by *C. perfringens*
- **clusters of differentiation (CD)** cell-surface glycoproteins that serve to identify and distinguish white blood cells
- **coagulase** enzyme that causes the activation of fibrinogen to form fibrin, promoting clotting of the blood
- **coarse focusing knob** a knob on a microscope that produces relatively large movements to adjust focus
- **coccidioidomycosis** disease caused by the highly infectious fungal pathogen *Coccidioides immitis* and related species
- **codon** three-nucleotide sequence within mRNA that specifies a particular amino acid to be incorporated into the polypeptide being synthesized
- **coenocyte** multinucleated eukaryotic cell that forms as a result of multiple rounds of nuclear division without the accompanying division of the plasma membrane
- **coenocytic hyphae** nonseptate hyphae that are multinucleate and lack cell walls or membranes between cells; characteristic of some fungi
- **coenzyme** organic molecule required for proper enzyme function that is not consumed and is reusable
- **cofactor** inorganic ion that helps stabilize enzyme conformation and function

- **cognate amino acid** amino acid added to a specific tRNA molecule that correctly corresponds to the tRNA's anticodon and, hence, the mRNA's codon, reflecting the genetic code
- **cohort method** a method used in observational studies in which a group of individuals is followed over time and factors potentially important in the development of disease are evaluated
- **colistin** membrane-active polymyxin that was historically used for bowel decontamination but now used for systemic infections with drug-resistant pathogens
- **colitis** inflammation of the large intestine
- **collagenase** enzyme that digests collagen, the dominant protein in connective tissue
- **colony-forming unit (CFU)** a counting quantity represented by a colony formed on solid medium from a single cell or a few cells
- **commensalism** type of symbiosis in which one population benefits and the other is not affected
- **commercial sterilization** type of sterilization protocol used in food production; uses conditions that are less harsh (lower temperatures) to preserve food quality but still effectively destroy vegetative cells and endospores of common foodborne pathogens such as *Clostridium botulinum*
- **common cold** most common cause of rhinitis in humans; associated with a variety of adenoviruses, coronaviruses, and rhinoviruses
- **common source spread** a mode of disease transmission in which every infection originates from the same source
- **communicable** able to be transmitted directly or indirectly from one person to another
- **community** group of interacting populations of organisms
- **competitive inhibitor** molecule that binds to an enzyme's active site, preventing substrate binding
- **competitive interactions** interactions between populations in which one of them competes with another for resources
- **complement activation** cascading activation of the complement proteins in the blood, resulting in opsonization, inflammation, and lysis of pathogens
- **complement fixation test** test for antibodies against a specific pathogen using complement-mediated hemolysis

- **complement system** series of proteins that can become activated in the presence of invading microbes, resulting in opsonization, inflammation, and lysis of pathogens
- **complementary base pairs** base pairing due to hydrogen bonding that occurs between a specific purine and a specific pyrimidine; A bonds with T (in DNA), and C bonds with G
- **complementary DNA (cDNA)** a DNA molecule complementary to mRNA that is made through the activity of reverse transcriptase
- **complex media** media that contain extracts of animals and plants that are not chemically defined
- **complex virus** virus shape that often includes intricate characteristics not seen in the other categories of capsid
- **compound microscope** a microscope that uses multiple lenses to focus light from the specimen
- **condenser lens** a lens on a microscope that focuses light from the light source onto the specimen
- **conditional mutation** mutant form of a gene whose mutant phenotype is expressed only under certain environmental conditions
- **confocal microscope** a scanning laser microscope that uses fluorescent dyes and excitation lasers to create three-dimensional images
- **conidia** asexual fungal spores not enclosed in a sac; produced in a chain at the end of specialized hyphae called conidiophores
- **conjugate vaccine** a vaccine consisting of a polysaccharide antigen conjugated to a protein to enhance immune response to the polysaccharide; conjugate vaccines are important for young children who do not respond well to polysaccharide antigens
- **conjugated protein** protein carrying a nonpolypeptidic portion
- **conjugation** mechanism of horizontal gene transfer in bacteria in which DNA is directly transferred from one bacterial cell to another by a conjugation pilus
- **conjugation pilus (sex pilus)** hollow tube composed of protein encoded by the conjugation plasmid that brings two bacterial cells into contact with each other for the process of conjugation
- **conjunctiva** the mucous membranes covering the eyeball and inner eyelid
- **conjunctivitis** inflammation of the conjunctiva, the mucous membrane covering the eye and inside of the eyelid

- **constitutively expressed** describes genes that are transcribed and translated continuously to provide the cell with constant intermediate levels of the protein products
- **contact dermatitis** inflammation of the skin resulting from a type IV hypersensitivity to an allergen or irritant
- **contact** see *exposure*
- **contact transmission** movement of a pathogen between hosts due to contact between the two; may be direct or indirect
- **contagious** easily spread from person to person
- **continuous cell line** derived from transformed cells or tumors, these cells are often able to be subcultured many times, or, in the case of immortal cell lines, grown indefinitely
- **continuous common source spread** a mode of disease transmission in which every infection originates from the same source and that source produces infections for longer than one incubation period
- **contractile vacuoles** organelles found in some cells, especially in some protists, that take up water and then move the water out of the cell for osmoregulatory purposes (i.e., to maintain an appropriate salt and water balance)
- **contrast** visible differences between parts of a microscopic specimen
- **convalescence stage** the final stage of a whooping cough infection, marked by a chronic cough
- **Coombs' reagent** antiserum containing antihuman immunoglobulins used to facilitate hemagglutination by cross-linking the human antibodies attached to red blood cells
- **cooperative interactions** interactions between populations in which both benefit
- **cortex** tightly packed layer of fungal filaments at the outer surface of a lichen; foliose lichens have a second cortex layer beneath the medulla
- **counterstain** a secondary stain that adds contrasting color to cells from which the primary stain has been washed out by a decolorizing agent
- **crenation** shriveling of a cell
- **Creutzfeldt-Jakob disease** form of transmissible spongiform encephalopathy found in humans; typically a fatal disease
- **crisis phase** point at which a fever breaks, reaching a peak before the hypothalamus resets back to normal body temperature

- **critical item** object that must be sterile because it will be used inside the body, often penetrating sterile tissues or the bloodstream
- **cross-match** in the major cross-match, donor red blood cells are checked for agglutination using recipient serum; in the minor cross-match, donor serum is checked for agglutinating antibodies against recipient red blood cells
- **cross-presentation** a mechanism by which dendritic cells process antigens for MHC I presentation to CD8 T cells through phagocytosis of the pathogen (which would normally lead to MHC II presentation)
- **cross-resistance** when a single resistance mechanism confers resistance to multiple antimicrobial drugs
- **cross-sectional study** a type of observational study in which measurements are made on cases, both affected and unaffected, at one point in time and the measurements analyzed to uncover associations with the disease state
- **crustose lichens** lichens that are tightly attached to the substrate, giving them a crusty appearance
- **cryptococcosis** fungal pneumonia caused by the encapsulated yeast *Cryptococcus neoformans* commonly found in bird droppings
- **cryptosporidiosis** intestinal infection caused by *Cryptosporidium parvum* or *C. hominis*
- **culture density** the number of cells per volume of broth
- **culture medium** combination of compounds in solution that supports growth
- **cutaneous mycosis** any fungal infection that affects the surface of the skin, hair, or nails
- **cyanobacteria** phototrophic, chlorophyll-containing bacteria that produce large amounts of gaseous oxygen
- **cyclic AMP (cAMP)** intracellular signaling molecule made through the action of adenylyl cyclase from ATP when glucose levels are low, with the ability to bind to a catabolite activator protein to allow it to bind to regulatory regions and activate the transcription of operons encoding enzymes for metabolism of alternative substrates
- **cyclic photophosphorylation** pathway used in photosynthetic organisms when the cell's need for ATP outweighs that for NADPH, thus bypassing NADPH production
- **cyclosporiasis** intestinal infection caused by *Cyclospora cayetanensis*

- **cystic echinococcosis** hydatid disease, an infection caused by the tapeworm *Echinococcus granulosus* that can cause cyst formation
- **cysticerci** larval form of a tapeworm
- **cystitis** inflammation of the bladder
- **cysts** microbial cells surrounded by a protective outer covering; some microbial cysts are formed to help the microbe survive harsh conditions, whereas others are a normal part of the life cycle
- **cytochrome oxidase** final ETS complex used in aerobic respiration that transfers energy-depleted electrons to oxygen to form H₂O
- **cytokine storm** an excessive release of cytokines, typically triggered by a superantigen, that results in unregulated activation of T cells
- **cytokines** protein molecules that act as a chemical signals; produced by cells in response to a stimulation event
- **cytomegalovirus (CMV) infection** human herpesvirus 5 infection that is typically asymptomatic but can become serious in immunocompromised patients, transplant recipients, and developing fetuses
- **cytopathic effect** cell abnormality resulting from a viral infection
- **cytoplasm** the gel-like material composed of water and dissolved or suspended chemicals contained within the plasma membrane of a cell
- **cytoplasmic membrane** see *cell membrane*
- **cytoproct** a protozoan cell structure that is specialized for excretion
- **cytosine** pyrimidine nitrogenous base found in nucleotides
- **cytoskeleton** a network of filaments or tubules in the eukaryotic cell that provides shape and structural support for cells; aids movement of materials throughout the cell
- **cytostome** a protozoan cell structure that is specialized for phagocytosis (i.e., to take in food)
- **cytotoxic T cells** effector cells of cellular immunity that target and eliminate cells infected with intracellular pathogens through induction of apoptosis
- **cytotoxicity** harmful effects to host cell

- **dacryocystitis** inflammation of the lacrimal sac often associated with a plugged nasolacrimal duct
- **daptomycin** cyclic lipopeptide that disrupts the bacterial cell membrane
- **darkfield microscope** a compound light microscope that produces a bright image on a dark background; typically a modified brightfield microscope
- **death phase (decline phase)** phase of the growth curve at which the number of dying cells exceeds the number of new cells formed
- **decimal reduction time (DRT) or D-value** amount of time it takes for a specific protocol to produce a one order of magnitude decrease in the number of organisms; that is, death of 90% of the population
- **decolorizing agent** a substance that removes a stain, usually from some parts of the specimen
- **deeply branching bacteria** bacteria that occupy the lowest branches of the phylogenetic tree of life
- **definitive host** the preferred host organism for a parasite, in which the parasite reaches maturity and may reproduce sexually
- **degeneracy** redundancy in the genetic code because a given amino acid is encoded by more than one nucleotide triplet codon
- **degerming** protocol that significantly reduces microbial numbers by using mild chemicals (e.g., soap) and gentle scrubbing of a small area of skin or tissue to avoid the transmission of pathogenic microbes
- **degranulation** release of the contents of mast cell granules in response to the cross-linking of IgE molecules on the cell surface with allergen molecules
- **dehydration synthesis** chemical reaction in which monomer molecules bind end to end in a process that results in the formation of water molecules as a byproduct
- **deletion** type of mutation involving the removal of one or more bases from a DNA sequence
- **Deltaproteobacteria** class of Proteobacteria that includes sulfate-reducing bacteria
- **denatured protein** protein that has lost its secondary and tertiary structures (and quaternary structure, if applicable) without the loss of its primary structure
- **dendrites** branched extensions of the soma of a neuron that interact with other cells

- **dengue fever** mosquito-borne viral hemorrhagic disease; also known as breakbone fever
- **dental calculus** calcified heavy plaque on teeth, also called tartar
- **dental caries** cavities formed in the teeth as a result of tooth decay caused by microbial activity
- **deoxyribonucleic acid (DNA)** double-stranded nucleic acid composed of deoxyribonucleotides that serves as the genetic material of the cell
- **deoxyribonucleotides** DNA nucleotides containing deoxyribose as the pentose sugar component
- **dermatophyte** any fungus of the genera *Microsporum*, *Epidermophyton*, or *Trichophyton*, which feed on keratin (a protein found in skin, hair, and nails) and can cause cutaneous infections
- **dermis** the second layer of human skin, found between the epidermis and the hypodermis
- **descriptive epidemiology** a method of studying a disease outbreak using case histories, contact interviews, medical information, and other sources of information
- **desensitization** injections of antigen that lead to production of antigen-specific IgG molecules, effectively outcompeting IgE molecules on the surface of sensitized mast cells for antigen
- **desiccation** method of microbial control involving the removal of water from cells through drying or dehydration
- **desquamation** peeling and shedding of outermost skin
- **diapedesis** process by which leukocytes pass through capillary walls to reach infected tissue; also called extravasation
- **diaphragm** a component of a microscope; typically consists of a disk under the stage with holes of various sizes; can be adjusted to allow more or less light from the light source to reach the specimen
- **differential interference-contrast microscope** a microscope that uses polarized light to increase contrast
- **differential media** media that contain additives that make it possible to distinguish bacterial colonies based on metabolic activities of the organisms
- **differential staining** staining that uses multiple dyes to differentiate between structures or organisms
- **diffraction** the changing of direction (bending or spreading) that occurs when a light wave interacts with an opening or barrier

- **dikaryotic** having two separate nuclei within one cell
- **dimorphic fungus** a fungus that can take the form of a yeast or a mold, depending on environmental conditions
- **dioecious** refers to sexually reproducing organisms in which individuals have either male or female reproductive organs (not both)
- **diphtheria** serious infection of the larynx, caused by the toxigenic bacterium *Corynebacterium diphtheriae*
- **diploid** having two copies of each chromosome
- **direct agglutination assay** assay that can be used to detect the agglutination of bacteria by the action of antibodies in patient serum
- **direct antihuman globulin test (DAT)** another name for a direct Coombs' test
- **direct contact transmission** movement of a pathogen between hosts by physical contact or transfer in droplets at a distance less than one meter
- **direct Coombs' test** assay that looks for antibodies *in vivo* against red blood cells caused by various types of infections, drug reactions, and autoimmune disorders
- **direct ELISA** enzyme-linked immunoabsorbent assay in which the antigens are immobilized in the well of a microtiter plate; only a single antibody is used in the test
- **direct fluorescent antibody (DFA) test** FA technique in which the labeled antibody binds to the target antigen
- **direct hemagglutination assay** test that determines the titer of certain bacteria and viruses that causes clumping of red blood cells
- **direct microscopic cell count** counting of cells using a calibrated slide under a light microscope
- **direct repair (light repair or photoreactivation)** light-dependent mechanism for repairing pyrimidine dimers involving the enzyme photolyase
- **disaccharide** one of two monosaccharides linked together by a glycosidic bond
- **disease** any condition in which the normal structure or function of the body is damaged or impaired
- **disinfectant** antimicrobial chemical applied to a fomite during disinfection that may be toxic to tissues
- **disinfection** protocol that removes potential pathogens from a fomite

- **disk-diffusion method** a technique for measuring of the effectiveness of one or more antimicrobial agents against a known bacterium; involves measuring the zone(s) of inhibition around the chemical agent(s) in a culture of the bacterium
- **dispersion** the separation of light of different frequencies due to different degrees of refraction
- **disulfide bridge** covalent bond between the sulfur atoms of two sulfhydryl side chains
- **DNA gyrase (topoisomerase II)** bacterial topoisomerase that relaxes the supercoiled chromosome to make DNA more accessible for the initiation of replication
- **DNA ligase** enzyme that catalyzes the formation of a covalent phosphodiester linkage between the 3'-OH end of one DNA fragment and the 5' phosphate end of another DNA fragment
- **DNA packaging** process in which histones or other DNA binding proteins perform various levels of DNA wrapping and attachment to scaffolding proteins to allow the DNA to fit inside a cell
- **DNA polymerase** class of enzymes that adds nucleotides to the free 3'-OH group of a growing DNA chain that are complementary to the template strand
- **DNA primers** short, synthetic, single-stranded DNA fragments of known sequence that bind to specific target sequences within a sample due to complementarity between the target DNA sequence and the primer; commonly used in PCR but may be used in other hybridization techniques
- **DNA probe** a single-stranded DNA fragment that is complementary to part of the gene (DNA or RNA) of interest
- **DNase** pathogen-produced nuclease that degrades extracellular DNA
- **dosage** amount of medication given during a certain time interval
- **double immunodiffusion** see Ouchterlony assay
- **doubling time** the time it takes for the population to double; also referred to as generation time
- **droplet transmission** direct contact transmission of a pathogen transferred in sneezed or coughed droplets of mucus that land on the new host within a radius of one meter
- **drug resistance** ability of a microbe to persist and grow in the presence of an antimicrobial drug

- **dry-heat sterilization** protocol that involves the direct application of high heat
- **dura mater** tough, outermost membrane that surrounds the brain
- **dynein** motor proteins that interact with microtubules in eukaryotic flagella and cilia
- **dysentery** intestinal inflammation that causes diarrhea with blood and mucus
- **dysuria** urination accompanied by burning, discomfort, or pain

E

- **E (exit) site** functional site of an intact ribosome that releases dissociated uncharged tRNAs so that they can be recharged with free amino acids
- **East African trypanosomiasis** acute form of African trypanosomiasis caused by *Trypanosoma brucei rhodesiense*
- **eastern equine encephalitis** serious, but rare, mosquito-borne viral infection of the brain that is found primarily on the Atlantic and Gulf coast states of the United States
- **Ebola virus disease (EVD)** potentially fatal viral hemorrhagic fever found primarily in western Africa and transmitted through contact with body fluids
- **eclipse phase** period after viral infection during which the infective virus is not detected, either intracellularly or extracellularly, and biosynthesis is occurring
- **ectoplasm** outer, more gelatinous layer of cytoplasm under a protist cell membrane
- **edema** swelling due to accumulation of fluid and protein in tissue as a result of increased permeability of capillary walls during an inflammatory response; chronic edema can also result from blockage of lymphatic vessels, as in the case of elephantiasis
- **effector cells** activated cells of cellular immunity that are involved in the immediate immune response, primarily to defend the body against pathogens
- **electron carrier** cellular molecule that accepts high-energy electrons from reduced molecules like foods and later serves as an electron

donor in subsequent redox reactions

- **electron microscope** a type of microscope that uses short-wavelength electron beams rather than light to increase magnification and resolution
- **electron transport system (ETS)** series of membrane-associated protein complexes and associated mobile accessory electron carriers important in the generation of the proton motive force required for ATP production by chemiosmosis; the last component involved in the cellular respiration of glucose
- **electroporation** a genetic engineering technique in which cells are exposed to a short electric pulse, inducing them to take up DNA molecules from their environment
- **elementary bodies** metabolically and reproductively inactive, endospore-like form of intracellular bacteria that spreads infection outside of cells
- **elongation in DNA replication** stage of DNA replication during which DNA polymerase adds nucleotides, complementary to the parental strand, to the 3' end of a growing DNA strand
- **elongation in transcription** stage of transcription during which RNA polymerase extends the RNA molecule by adding RNA nucleotides, complementary to the template DNA strand
- **elongation of translation** stage of translation during which amino acids are added one by one to the C-terminus of the growing polypeptide
- **Embden-Meyerhof-Parnas (EMP) pathway** type of glycolysis found in animals and the most common in microbes
- **emerging infectious disease** a disease that is new to the human population or has increased in prevalence over the previous 20 years
- **enantiomers** stereoisomers that are mirror images of each other and nonsuperimposable
- **encephalitis** inflammation of the tissues of the brain
- **encystment** the process of forming a cyst
- **endemic disease** an illness that is constantly present (often at low levels) in a population
- **endergonic reaction** chemical reaction that requires energy beyond activation energy to occur

- **endocarditis** inflammation of the endocardium, especially the heart valves
- **endocrine function** refers to a cytokine signal released from a cell and carried by the bloodstream to a distant recipient cell
- **endocytosis** the uptake of molecules through plasma membrane invagination and vacuole/vesicle formation
- **endomembrane system** a series of organelles (endoplasmic reticulum, Golgi apparatuses, lysosomes, and transport vesicles) arranged as membranous tubules, sacs, and disks that synthesize many cell components
- **endoplasm** inner, more fluid layer of cytoplasm under a protist cell membrane (inside of the ectoplasm)
- **endoplasmic reticulum** part of the endomembrane system that is an interconnected array of tubules and flattened sacs with a single lipid bilayer that may be either rough or smooth; important in synthesizing proteins and lipids
- **endospore** a cellular structure formed by some bacteria in response to adverse conditions; preserves DNA of the cell in a dormant state until conditions are favorable again
- **endospore staining** a differential staining technique that uses two stains to make bacterial endospores appear distinct from the rest of the cell
- **endosymbiotic theory** the theory that mitochondria and chloroplasts arose as a result of prokaryotic cells establishing a symbiotic relationship within a eukaryotic host
- **endothelia** layer of epithelial cells lining blood vessels, lymphatics, the blood-brain barrier, and some other tissues
- **endotoxin** lipid A component of lipopolysaccharides in the outer membrane of gram-negative bacteria
- **enriched media** media that contain additional essential nutrients to support growth
- **enrichment culture** media providing growth conditions that favor the expansion of an organism present in low numbers
- **enteric** bacteria of the family Enterobacteriaceae, which live in the human intestinal tract
- **enteritis** inflammation of the lining of the intestine

- **enterobiasis** intestinal infection caused by the pinworm *Enterobius vermicularis*
- **enterohemorrhagic *E. coli* (EHEC)** *E. coli* bacteria that cause severe gastrointestinal illness with potential serious complications such as hemolytic uremic syndrome
- **enteroinvasive *E. coli* (EIEC)** *E. coli* bacteria that cause relatively mild gastrointestinal illness
- **enteropathogenic *E. coli* (EPEC)** *E. coli* bacteria that cause serious gastrointestinal illness
- **enterotoxigenic *E. coli* (ETEC)** *E. coli* bacteria that cause a relatively mild illness commonly called traveler's diarrhea
- **enterotoxin** toxin that affects the intestines
- **Entner-Doudoroff (ED) pathway** alternative glycolytic pathway used by some bacteria
- **enveloped virus** a virus formed with a nucleic-acid packed capsid surrounded by a lipid layer
- **enzyme** catalyst for biochemical reactions inside cells
- **enzyme immunoassay (EIA)** type of assay wherein an enzyme is coupled to an antibody; addition of a chromogenic substrate for the antibody allows quantification or identification of the antigen bound by the antibody
- **enzyme-linked immunosorbent assay (ELISA)** specialized form of EIA in which either the primary antibody or the antigen is first attached to a solid surface such as the well of a microtiter plate
- **eosinophils** leukocytes with granules containing histamine and major basic protein; facilitate allergic responses and protection against parasitic protozoa and helminths
- **epidemic disease** an illness with a higher-than-expected incidence in a given period within a given population
- **epidemic typhus** severe and sometimes fatal infection caused by *Rickettsia prowazekii* and transmitted by body lice
- **epidemiology** the study of where and when infectious diseases occur in a population and how they are transmitted and maintained in nature
- **epidermis** the outermost layer of human skin
- **epididymis** coiled tube that collects sperm from the testes and passes it on to the vas deferens

- **epididymitis** inflammation of the epididymis caused by a bacterial infection
- **epigenetic regulation** chemical modification of DNA or associated histones to influence transcription
- **epiglottis** flap of cartilage that covers the larynx during swallowing; diverts food to the esophagus and prevents it from entering the respiratory tract
- **epiglottitis** inflammation of the epiglottis
- **epiphyte** a plant that grows on another plant
- **epitope** smaller exposed region on an antigen that is recognized by B-cell and T-cell receptors and antibodies
- **Epsilonproteobacteria** class of Proteobacteria that are microaerophilic
- **equivalence zone** region where the antibody–antigen ratio produces the greatest amount of precipitin in a precipitin reaction
- **erysipelas** a skin infection, typically caused by *Streptococcus pyogenes*, that presents as a red, large, intensely inflamed patch of skin involving the dermis, usually with clear borders, typically on the legs or face
- **erythema nodosum** a condition that causes inflammation in the subcutaneous fat cells of the hypodermis resulting in red nodules
- **erythema** redness at the site of inflammation, usually due to dilation of blood vessels in the area to help bring in white blood cells
- **erythrocyte** red blood cell
- **erythrogenic toxin** exotoxin produced by some strains of *Streptococcus pyogenes*; activity of the toxin can produce the characteristic rash of scarlet fever
- **erythromycin** protein synthesis inhibitor of the macrolide class that is often used as an alternative to penicillin
- **eschar** a localized mass of dead skin tissue
- **Etest** simple, rapid method for determining MIC, involving commercially available plastic strips that contain a gradient of an antimicrobial and are placed on an agar plate inoculated with a bacterial lawn
- **etiologic agent** the pathogen or substance responsible for causing a particular disease; causative agent
- **etiology** the science of the causes of disease

- **Eukarya** the domain of life that includes all unicellular and multicellular organisms with cells that contain membrane-bound nuclei and organelles
- **eukaryote** an organism made up of one or more cells that contain a membrane-bound nucleus and organelles
- **eukaryotic cell** has a nucleus surrounded by a complex nuclear membrane that contains multiple, rod-shaped chromosomes
- **eustachian tube** small passage between the nasopharynx and the middle ear that allows pressure to equalize across the tympanic membrane
- **eutrophs** microorganisms that require a copious amount of organic nutrients; also called copiotrophs
- **excystment** the process of emerging from a cyst
- **exergonic reaction** chemical reaction that does not require energy beyond activation energy to proceed; releases energy when the reaction occurs
- **exocytosis** the release of the contents of transport vesicles to the cell's exterior by fusion of the transport vesicle's membrane with the plasma membrane
- **exoenzyme** secreted enzyme that enhances the ability of microorganisms to invade host cells
- **exon** protein-coding sequence of a eukaryotic gene that is transcribed into RNA and spliced together to code for a polypeptide
- **exonuclease** enzymatic activity that removes RNA primers in DNA introduced by primase
- **exotoxin** biologically active product that causes adverse changes in the host cells
- **experimental epidemiology** the use of laboratory and clinical studies to directly study disease in a population
- **experimental study** a type of scientific study that involves manipulation of the study subjects by the researcher through application of specific treatments hypothesized to affect the outcome while maintaining rigorously controlled conditions
- **exposure** contact between potential pathogen and host; also called contamination or contact
- **extended-spectrum β -lactamases (ESBLs)** β -lactamases carried by some gram-negative bacteria that provide resistance to all penicillins,

cephalosporins, monobactams, and β -lactamase-inhibitor combinations, but not carbapenems

- **extensively drug resistant *Mycobacterium tuberculosis* (XDR-TB)** strains of *M. tuberculosis* that are resistant to rifampin and isoniazid, and also are resistant to any fluoroquinolone and at least one of three other drugs (amikacin, kanamycin, or capreomycin)
- **extracellular matrix** material composed of proteoglycans and fibrous proteins secreted by some eukaryotic cells that lack cell walls; helps multicellular structures withstand physical stresses and coordinates signaling from the external surface of the cell to the interior of the cell
- **extracellular polymeric substances (EPS)** hydrated gel secreted by bacteria in a biofilm containing polysaccharides, proteins, nucleic acids, and some lipids
- **extrachromosomal DNA** additional molecules of DNA distinct from the chromosomes that are also part of the cell's genome
- **extravasation** process by which leukocytes pass through capillary walls to reach infected tissue; also called diapedesis

F

- **F⁻ (recipient) cell** *E. coli* cell lacking the F plasmid and thus incapable of forming a conjugation pilus but capable of receiving the F plasmid during conjugation
- **F pilus (F pili)** specialized type of pilus that aids in DNA transfer between cells; conjugation pilus of *E. coli*
- **F plasmid (fertility factor)** bacterial plasmid in *E. coli* containing genes encoding the ability to conjugate, including genes encoding the formation of the conjugation pilus
- **F' plasmid** integrated F plasmid imprecisely excised from the chromosome; carries with it some chromosomal DNA adjacent to the integration site
- **F⁺ (donor) cell** *E. coli* cell containing the F plasmid, capable of forming a conjugation pilus
- **Fab region** arm of an antibody molecule that includes an antigen-binding site

- **facultative anaerobe** organism that grows better in the presence of oxygen but can proliferate in its absence
- **false negative** negative result to a test for an infection or condition (e.g., presence of antigen, antibody, or nucleic acid) when the infection or condition is actually present
- **false positive** positive result to a test for an infection or condition (e.g., presence of antigen, antibody, or nucleic acid) when the infection or condition is actually absent
- **fastidious organism** organism that has extensive growth requirements
- **fatty acid** lipid that contains long-chain hydrocarbons terminated with a carboxylic acid functional group
- **fatty acid methyl ester (FAME) analysis** technique in which the microbe's fatty acids are extracted, converted to volatile methyl esters, and analyzed by gas chromatography, yielding chromatograms that may be compared to reference data for identification purposes
- **Fc region** region on the trunk of an antibody molecule involved in complement activation and opsonization
- **feedback inhibition** mechanism of regulating metabolic pathway whereby the product of a metabolic pathway noncompetitively binds to an enzyme early on in the pathway, temporarily preventing the synthesis of the product
- **fermentation** process that uses an organic molecule as a final electron acceptor to regenerate NAD^+ from NADH such that glycolysis can continue
- **fever** system-wide sign of inflammation that raises the body temperature and stimulates the immune response
- **fifth disease** a highly contagious illness, more commonly affecting children, marked by a distinctive "slapped-cheek" rash and caused by parvovirus B19
- **fimbriae** filamentous appendages found by the hundreds on some bacterial cells; they aid adherence to host cells
- **fine focusing knob** a knob on a microscope that produces relatively small movements to adjust focus
- **fixation** the process by which cells are killed and attached to a slide
- **flagella** long, rigid, spiral structures used by prokaryotic cells for motility in aqueous environments; composed of a filament made of

flagellin, a hook, and motor (basal body) that are attached to the cell envelope

- **flagella staining** a staining protocol that uses a mordant to coat the flagella with stain until they are thick enough to be seen
- **flagellum (eukaryotic) (plural: flagella)** long, whip-like, filamentous external structure found on some eukaryotic cells; composed of microtubules in a 9+2 arrangement; used for locomotion
- **flavin adenine dinucleotide (FAD/FADH₂)** oxidized/reduced forms of an electron carrier in cells
- **flocculant** visible aggregation that forms between a substance in suspension (e.g., lipid in water) and antibodies against the substance
- **flow cytometry** technique analyzing cells for fluorescence intensity; specific subsets of cells are usually labeled in some way prior to the analysis
- **fluconazole** antifungal drug of the imidazole class that is administered orally or intravenously for the treatment of several types of systemic yeast infections
- **fluid mosaic model** refers to the ability of membrane components to move fluidly within the plane of the membrane, as well as the mosaic-like composition of the components
- **flukes** any of the parasitic nonsegmented flatworms (trematodes) that have an oral sucker and sometimes a second ventral sucker; they attach to the inner walls of intestines, lungs, large blood vessels, or the liver in human hosts
- **fluorescence microscope** a microscope that uses natural fluorochromes or fluorescent stains to increase contrast
- **fluorescence-activated cell sorter (FACS)** technique for using a flow cytometer to physically separate cells into two populations based on fluorescence intensity
- **fluorescent antibody (FA) techniques** suite of assays that use a fluorescently labeled antibody to bind to and so make an antigen easy to visualize
- **fluorescent enzyme immunoassay (FEIA)** EIA in which the substrate is a fluorogen that becomes fluorescent following reaction with the enzyme
- **fluorescent** the ability of certain materials to absorb energy and then immediately release that energy in the form of light

- **fluorochromes** chromophores that fluoresce (absorb and then emit light)
- **fluorogen** nonfluorescent molecule that becomes fluorescent on enzyme or laser activation
- **fluorophore** molecule that fluoresces when excited by light
- **fluoroquinolones** class of synthetic antimicrobials that inhibit the activity of DNA gyrase, preventing DNA replication
- **focal infection** infection in which the pathogen causes infection in one location that then spreads to a secondary location
- **focal length** the distance from the lens to the image point when the object is at a definite distance from the lens (this is also the distance to the focal point)
- **focal point** a property of the lens; the image point when light entering the lens is parallel (i.e., the object is an infinite distance from the lens)
- **foliose lichens** lichens that have lobes that may appear to resemble leaves
- **folliculitis** a skin infection characterized by localized inflammation of hair follicles, typically producing an itchy red rash
- **fomite** inanimate item that may harbor microbes and aid in disease transmission
- **foodborne disease** disease that is transmitted through contaminated food
- **fragmentation** newly formed cells split away from the parent filament in actinomycetes and cyanobacteria
- **frameshift mutation** mutation resulting from either an insertion or a deletion in a number of nucleotides that, if not a multiple of three, changes every amino acid after the mutation
- **free ribosome** eukaryotic 80S ribosome found in the cytoplasm; synthesizes water-soluble proteins
- **frequency** the rate of vibration for a light wave or other electromagnetic wave
- **fruticose lichens** lichens that are generally branched with a rounded appearance
- **functional groups** specific groups of atoms that may occur within a molecule, conferring specific chemical properties
- **fungi** (singular: fungus) any of various unicellular or multicellular eukaryotic organisms, typically having cell walls made out of chitin

- and lacking photosynthetic pigments, vascular tissues, and organs
- **fungicide** chemical or physical treatment that kills fungi
- **fungistatic** having the ability to inhibit fungal growth, generally by means of chemical or physical treatment
- **furuncle** a small, purulent skin lesion; sometimes called a boil
- **fusion inhibitor** antiviral drug that blocks the fusion of HIV receptors to the coreceptors required for virus entry into the cell, specifically, chemokine receptor type 5

G

- **Gammaproteobacteria** class of Proteobacteria that is very diverse and includes a number of human pathogens
- **gas gangrene** rapidly spreading infection of necrotic tissues caused by the gram-positive anaerobe *Clostridium perfringens* and other *Clostridium* spp.
- **gastritis** inflammation of the lining of the stomach
- **gastroenteritis** inflammation of the lining of the stomach and intestine
- **gene expression** production of proteins from the information contained in DNA through the processes of transcription and translation
- **gene gun** an apparatus that shoots gold or tungsten particles coated with recombinant DNA molecules at high speeds into plant protoplasts
- **gene silencing** a genetic engineering technique in which researchers prevent the expression of a particular gene by using small interfering RNAs (siRNAs) or microRNAs (miRNAs) to interfere with translation
- **gene therapy** a form of treatment for diseases that result from genetic mutations; involves the introduction of nonmutated, functional genes into the genome of the patient, often by way of a viral vector
- **generalized transduction** transfer of a random piece of bacterial chromosome DNA by the phage
- **generation time** see *doubling time*
- **genes** segments of DNA molecules that code for proteins or stable RNA molecules
- **genetic code** correspondence between mRNA nucleotide codons and the translated amino acids

- **genetic engineering** the direct alteration of an organism's genetics to achieve desirable traits
- **genital herpes** an STI caused by the herpes simplex virus
- **genital warts** soft, pink, irregular growths that develop in the external genitalia or anus as a result of human papillomavirus infection
- **genome** entire genetic content of a cell
- **genomic library** a repository of an organism's entire genome maintained as cloned fragments in the genomes of strains of a host organism
- **genomics** the study and comparison of entire genomes, including the complete set of genes, their nucleotide sequence and organization, and their interactions within a species and with other species
- **genotype** full collection of genes that a cell contains within its genome
- **germ theory of disease** the theory that many diseases are the result of microbial infection
- **germination** process of an endospore returning to the vegetative state
- **Ghon complex** calcified lesion containing *Mycobacterium tuberculosis*; forms in the lungs of patients with tuberculosis
- **giardiasis** intestinal infection caused by *Giardia lamblia*
- **gingivitis** inflammation of the gums that can cause bleeding
- **glial cell** assists in the organization of neurons, provides a scaffold for some aspects of neuron function, and aids in recovery from neural injury
- **glomerulonephritis** a type of kidney infection involving the glomeruli of the nephrons
- **glomerulus** capillary bed in the nephron of the kidney that filters blood to form urine
- **glycocalyx** cell envelope structure (either capsules or slime layer) outside the cell wall in some bacteria; allows bacteria to adhere to surfaces, aids in biofilm formation, and provides protection from predation
- **glycogen** highly branched storage polysaccharide in animal cells and bacteria
- **glycolipid** complex lipid that contains a carbohydrate moiety
- **glycolysis** first step in the breakdown of glucose, the most common example of which is the Embden-Meyerhoff-Parnas pathway,

producing two pyruvates, two NADH molecules, and two (net yield) ATP per starting glucose molecule

- **glycopeptides** class of antibacterials that inhibit cell wall synthesis by binding to peptidoglycan subunits and blocking their insertion into the cell wall backbone, as well as blocking transpeptidation
- **glycoprotein** conjugated protein with a carbohydrate attached
- **glycosidic bond** forms between the hydroxyl groups of two sugar molecules
- **Golgi apparatus** an organelle of the endomembrane system that is composed of a series of flattened membranous disks, called dictyosomes, each having a single lipid bilayer, that are stacked together; important in the processing of lipids and proteins
- **gonorrhea** a common STI of the reproductive system caused by *Neisseria gonorrhoeae*
- **graft-versus-host disease** specific type of transplantation reaction in which a transplanted immune system (e.g., a bone marrow transplant) contains APCs and T cells that are activated and attack the recipient's tissue
- **Gram stain procedure** a differential staining technique that distinguishes bacteria based upon their cell wall structure
- **granulocytes** leukocytes found in the peripheral blood that are characterized by numerous granules in the cytoplasm; granulocytes include neutrophils, eosinophils, and basophils
- **granuloma** walled-off area of chronically inflamed tissue containing microbial pathogens, macrophages, and cellular materials unable to be eliminated
- **granulomatous amoebic encephalitis (GAE)** serious brain infection of immunocompromised individuals caused by *Acanthamoeba* or *Balamuthia mandrillaris*
- **granzymes** proteases released from a natural killer cell that enter the cytoplasm of a target cell, inducing apoptosis
- **Graves disease** hyperthyroidism caused by an autoimmune disease affecting thyroid function
- **green nonsulfur bacteria** similar to green sulfur bacteria but use substrates other than sulfides for oxidation
- **green sulfur bacteria** phototrophic, anaerobic bacteria that use sulfide for oxidation and produce large amounts of green bacteriochlorophyll

- **growth curve** a graph modeling the number of cells in a culture over time
- **guanine** purine nitrogenous base found in nucleotides
- **Guillain-Barré syndrome** an autoimmune disease, often triggered by bacterial and viral infections, characterized by the destruction of myelin sheaths around neurons, resulting in flaccid paralysis
- **gummas** granulomatous lesions that develop in tertiary syphilis

H

- **hair follicle** a structure embedded in the dermis from which hair grows
- **halophile** organism that depends on high concentrations of salt in the environment to grow
- **halotolerant** organism that grows in the presence of high salt concentration but does not require it
- **Hansen's Disease** chronic bacterial infection of peripheral nervous tissues caused by the acid-fast bacterium, *Mycobacterium leprae*; also known as leprosy
- **hantavirus pulmonary syndrome** acute lung infection by a hantavirus following inhalation of aerosols from the urine or feces of infected rodents
- **haploid** having one copy of each chromosome
- **haptin** a molecule that is too small to be antigenic alone but becomes antigenic when conjugated to a larger protein molecule
- **hard chancre** a generally painless ulcer that develops at the site of infection in primary syphilis
- **Hashimoto thyroiditis** hypothyroidism caused by an autoimmune disease affecting thyroid function
- **healthcare-associated infection (HAI)** an infection acquired in a hospital or other health-care facility unrelated to the reason for which the patient was initially admitted; nosocomial infection
- **heavy chains** longest identical peptide chains in antibody molecules (two per antibody monomer), composed of variable and constant region segments
- **helical virus** cylindrical or rod shaped

- **helicase** enzyme that unwinds DNA by breaking the hydrogen bonds between the nitrogenous base pairs, using ATP
- **helminth** a multicellular parasitic worm
- **helper T cells** class of T cells that is the central orchestrator of the cellular and humoral defenses of adaptive immunity and the cellular defenses of innate immunity
- **hemagglutination** visible clumping of red blood cells that can be caused by some viruses, bacteria, and certain diseases in which antibodies are produced that bind to self-red blood cells
- **hematopoiesis** formation, development, and differentiation of blood cells from pluripotent hematopoietic stem cells
- **hematuria** condition in which there is blood in the urine
- **hemolysin** class of exotoxin that targets and lyses red blood cells, as well as other cells
- **hemolytic disease of the newborn (HDN)** type II hypersensitivity reaction that occurs when maternal anti-Rh antibodies cross the placenta and target fetal Rh⁺ red blood cells for lysis
- **hemolytic transfusion reaction (HTR)** condition resulting after an incompatible blood transfusion; caused by type II hypersensitivity reaction and destruction of red blood cells
- **hemorrhagic fever with renal syndrome** serious hemorrhagic fever caused by hantavirus infection
- **HEPA filter** high-efficiency particulate air filter with an effective pore size that captures bacterial cells, endospores, and viruses as air passes through, removing them from the air
- **hepatitis** inflammation of the liver
- **herd immunity** a reduction in disease prevalence brought about when few individuals in a population are susceptible to an infectious agent
- **herpes keratitis** eye infection caused by herpes simplex virus
- **herpes simplex virus type 2 (HSV-2)** the type of herpesvirus most commonly associated with genital herpes
- **herpetic gingivostomatitis** inflammation of the mouth and gums often caused by the HSV-1 virus
- **heterolactic fermentation** process producing a mixture of lactic acid, ethanol and/or acetic acid, and CO₂ as fermentation products; the microbes that do this use pentose phosphate pathway glycolysis, which is why they generate multiple fermentation products

- **heterotroph** organism that uses fixed organic carbon compounds as its carbon source
- **hexose monophosphate shunt** see *pentose phosphate pathway*
- **Hfr cell** *E. coli* cell in which an F plasmid has integrated into the host cell's chromosome
- **high G+C gram-positive bacteria** bacteria that have more than 50% guanine and cytosine nucleotides in their DNA
- **high-energy phosphate bond** bond between the negatively charged phosphate groups that holds a lot of potential energy
- **histamine** proinflammatory molecule released by basophils and mast cells in response to stimulation by other cytokines and chemical mediators
- **histones** DNA-binding proteins found in eukaryotes and archaea that aid in orderly packaging of chromosomal DNA
- **histoplasmosis** fungal disease caused by the dimorphic fungus *Histoplasma capsulatum*
- **holoenzyme** enzyme with a bound cofactor or coenzyme
- **holozoic** refers to protozoans that consume food particles through phagocytosis
- **homolactic fermentation** process producing only lactic acid as a fermentation product; the microbes that do this use Embden-Meyerhof-Parnas glycolysis
- **hookworm infection** soil-transmitted intestinal infection caused by the nematodes *Necator americanus* and *Ancylostoma doudenale*
- **horizontal direct transmission** movement of a pathogen from one host to another (excluding mother to embryo, fetus, or infant) in a population through physical contact or through droplet transmission
- **horizontal gene transfer** introduction of genetic material from one organism to another organism within the same generation
- **host range** the types of host cells that a particular virus is able to infect
- **HTST** high-temperature short-time pasteurization is a method of pasteurization commonly used for milk in which the milk is exposed to a temperature of 72 °C for 15 seconds
- **human African trypanosomiasis** serious infection caused by *Trypanosoma brucei* and spread by the bite of the tsetse fly

- **human granulocytic anaplasmosis** zoonotic tickborne disease caused by the obligate intracellular pathogen *Anaplasma phagocytophilum*
- **human immunodeficiency virus (HIV)** retrovirus responsible for acquired immune deficiency syndrome (AIDS) in humans
- **human papillomavirus (HPV)** a group of common sexually transmitted viruses that may be associated with genital warts or with cervical cancer
- **humanized monoclonal antibodies** chimeric antibodies with mouse variable regions and human constant regions
- **humoral immunity** adaptive immunity mediated by antibodies produced by B cells
- **hyaluronidase** enzyme produced by pathogens that degrades hyaluronic acid between adjacent cells in connective tissue
- **hybridization** the joining of two complementary single-stranded DNA molecules
- **hybridoma** clones of cell produced by fusing a normal B cell with a myeloma cell that is capable of producing monoclonal antibodies indefinitely
- **hydatid disease** cystic echinococcosis, an infection caused by the tapeworm *Echinococcus granulosus*
- **hydrophilic** “water loving”; refers to a polar molecule or portion of a molecule capable of strong attraction to water molecules
- **hydrophobic** “water fearing”; refers to a nonpolar molecule or portion of a molecule not capable of strong attraction to water molecules
- **hypersensitivity pneumonitis (HP)** type III and IV hypersensitivities in the lungs that are caused by environmental or occupational exposure to allergens such as mold and dust
- **hypersensitivity** potentially damaging immune response against an antigen
- **hyperthermophile** a microorganism that has an optimum growth temperature close to the temperature of boiling water
- **hypertonic medium** an environment in which the solute concentration outside a cell exceeds that inside the cell, causing water molecules to move out of the cell, resulting in crenation (shriveling) or plasmolysis.
- **hyphae** tubular, filamentous structures that makes up most fungi
- **hypodermis** the layer of tissue under the dermis, consisting primarily of fibrous and adipose connective tissue

- **hypotonic medium** an environment in which the solute concentration inside a cell exceeds that outside the cell, causing water molecules to move into the cell, possibly leading to swelling and possibly lysis

I

- **iatrogenic disease** disease caused by or acquired during a medical procedure
- **icosahedral** three-dimensional, 20-sided structure with 12 vertices
- **IgA** antibody dimer primarily found in breast milk, mucus, saliva, and tears
- **IgD** membrane-bound antibody monomer functioning as receptor on the surface of B cells
- **IgE** antibody monomer involved in defense against parasites and allergic reactions
- **IgG** antibody monomer most abundant in serum; able to cross placenta; most versatile class of antibody in terms of function
- **IgM** antibody that is a monomer when functioning as a receptor on surface of B cells but a pentamer when secreted in response to specific pathogens; first antibody to respond during primary and secondary responses
- **illuminator** the light source on a microscope
- **image point (focus)** a property of the lens and the distance of the object to the lens; the point at which an image is in focus (the image point is often called the focus)
- **imidazoles** class of antifungal drugs that inhibit ergosterol biosynthesis
- **immune complex** large group of antigens bound by antibodies; large enough to settle out of fluid suspension
- **immunochromatographic assay** assay in which fluids are pulled through test strips by capillary action and antigen captured by mobile antibody-colored bead conjugates; a second, fixed antibody localizes the colored bead, allowing visualization
- **immunocytochemistry (ICC)** staining technique in which cells are fixed and holes dissolved in the membrane to allow passage of labeled antibodies to bind specific intracellular targets

- **immuno-electrophoresis (IEP)** assay following protein electrophoresis (PAGE) of serum, in which antisera against specific serum proteins are added to troughs cut parallel to the electrophoresis track, causing the formation of precipitin arcs
- **immunofiltration** technique in which antibody or antigen can be concentrated by passing fluids through porous membranes, and target molecules are captured as they pass
- **immunofluorescence** a technique that uses a fluorescence microscope and antibody-specific fluorochromes to determine the presence of specific pathogens in a specimen
- **immunoglobulin** antibody
- **immunohistochemistry (IHC)** staining technique in which labeled antibodies are bound to specific cells in a tissue section
- **immunology** the study of the immune system
- **immunostain** use of EIA technology to deliver stain to particular cells in a tissue (immunohistochemistry) or specific targets within a cell (immunocytochemistry)
- **impetigo** a skin infection that may result in vesicles, blisters, or bullae especially around the mouth, commonly caused by *Staphylococcus aureus*, *S. pyogenes*, or a combination of both *S. aureus* and *S. pyogenes*
- **in vitro** outside the organism in a test tube or artificial environment
- **in vivo** inside the organism
- **inactivated vaccine** vaccine composed of whole pathogen cells or viruses that have been killed or inactivated through treatment with heat, radiation, or chemicals
- **incidence** the number of individuals with new infections of a particular disease in a given period of time
- **inclusion conjunctivitis** inflammation of the conjunctiva in newborns caused by *Chlamydia trachomatis* transmitted during childbirth
- **inclusions** prokaryotic cell cytoplasmic structures for storing specific nutrients and other resources needed by cells
- **incubation period** the first stage of acute disease, during which the pathogen begins multiplying in the host and signs and symptoms are not observable
- **indirect agglutination assay** assay that can be used to detect the agglutination of small latex beads; beads may be coated with antigen

when looking for the presence of specific antibodies, or with antibody when looking for the presence of antigen

- **indirect antiglobulin test (IAT)** see indirect Coombs' test
- **indirect contact transmission** transfer of an infectious agent between hosts through contact with a fomite
- **indirect Coombs' test** assay, performed *in vitro* prior to blood transfusions, that looks for antibodies against red blood cell antigens (other than the A and B antigens) that are unbound in a patient's serum
- **indirect ELISA** EIA in which an antigen from a pathogen is first attached to the wells of a microtiter plate; the antigen then captures antibodies from patient serum to determine whether the patient currently has or previously had the disease
- **indirect fluorescent antibody test** assay for antigen-specific antibodies wherein the antigen captures the antibody, which is subsequently detected using a labeled anti-immunoglobulin mAb
- **induced mutation** mutation caused by a mutagen
- **inducer** small molecule that either activates or represses transcription
- **inducible operon** bacterial operon, typically containing genes encoding enzymes in a degradative pathway, whose expression is induced by the substrate to be degraded when the substrate is available for the cell to use, but that is otherwise repressed in the absence of the substrate
- **induction** prophage DNA is excised from the bacterial genome
- **infection** the successful colonization of a microorganism within a host
- **infectious arthritis (septic arthritis)** inflammation of joint tissues in response to a microbial infection
- **infectious disease** disease caused by a pathogen
- **infectious mononucleosis** common and mild infection caused by Epstein-Barr virus (HHV-4) or cytomegalovirus (HHV-5); transmitted by direct contact with body fluids such as saliva
- **inflammation** innate nonspecific immune response characterized by erythema, edema, heat, pain, and altered function, typically at the site of injury or infection but sometimes becoming systemic.
- **influenza** highly contagious and acute viral disease of the respiratory tract caused by the influenza virus
- **initiation factors** proteins that participate in ribosome assembly during initiation

- **initiation of DNA replication** stage of replication during which various proteins bind to the origin of replication to begin the replication process
- **initiation of transcription** stage of transcription during which RNA polymerase binds to a promoter and transcription begins
- **initiation of translation** stage of translation during which an initiation complex composed of the small ribosomal subunit, the mRNA template, initiation factors, GTP, and a special initiator tRNA forms, and the large ribosomal subunit then binds to the initiation complex
- **inoculum** small number of cells added to medium to start a culture
- **inorganic phosphate (P_i)** single phosphate group in solution
- **insertion** type of mutation involving the addition of one or more bases into a DNA sequence
- **integrase inhibitors** antiviral drugs that block the activity of the HIV integrase responsible for recombination of a DNA copy of the viral genome into the host cell chromosome
- **intercalating agent** molecule that slides between the stacked nitrogenous bases of the DNA double helix, potentially resulting in a frameshift mutation
- **interference** distortion of a light wave due to interaction with another wave
- **interferons** cytokines released by cells that have been infected with a virus; stimulate antiviral responses in nearby cells as well as the cells secreting the interferons
- **interleukins** cytokines largely produced by immune system cells that help coordinate efforts against invading pathogens
- **intermediate filament** one of a diverse group of cytoskeletal fibers that act as cables within the cell and anchor the nucleus, comprise the nuclear lamina, or contribute to the formation of desmosomes
- **intermediate host** a host in which a parasite goes through some stages of its life cycle before migrating to the definitive host
- **intermittent common source spread** a mode of disease transmission in which every infection originates from the same source and that source produces infections for a period before stopping and then starting again
- **intertrigo** a rash that occurs in a skin fold

- **intestinal fluke** a trematode worm that infects the intestine, often caused by *Fasciolopsis buski*
- **intracellular targeting toxin** see *A-B exotoxin*
- **intrinsic growth rate** genetically determined generation time under specific conditions for a bacterial strain
- **intron** intervening sequence of a eukaryotic gene that does not code for protein and whose corresponding RNA sequences are removed from the primary transcript during splicing
- **intubation** placement of a tube into the trachea, generally to open the airway or to administer drugs or oxygen
- **in-use test** a technique for monitoring the correct use of disinfectants in a clinical setting; involves placing used, diluted disinfectant onto an agar plate to see if microbial colonies will grow
- **invasion** dissemination of a pathogen through local tissues or throughout the body
- **iodophor** compound in which iodine is complexed to an organic molecule, increasing the stability and efficacy of iodine as a disinfectant
- **ionizing radiation** high-energy form of radiation that is able to penetrate surfaces and sterilize materials by damaging microbial cell components and DNA
- **ischemia** condition marked by the inadequate flow of blood to the tissues
- **isograft** tissue grafted from one monozygotic twin to another
- **isohemagglutinins** IgM class antibodies produced against A or B red blood cell antigens
- **isomers** molecules that have the same atomic makeup but differ in the structural arrangement of the atoms
- **isoniazid** antimetabolite that inhibits biosynthesis of mycolic acid; used for the treatment of mycobacterial infections
- **isoprenoid** branched lipid derived from five-carbon isoprene molecules
- **isotonic medium** a solution in which the solute concentrations inside and outside the cell are approximately equal, thereby creating no net movement of water molecules across the cell membrane
- **ivermectin** antihelminthic drug of the avermectin class that binds to invertebrate glutamate-gated chloride channels to block neuronal

transmission in helminths

J

- **Japanese encephalitis** arboviral disease caused by the Japanese encephalitis virus (JEV) and endemic to Asia
- **jaundice** yellowish color of the skin and mucous membranes caused by excessive bilirubin caused by a failure of the liver to effectively process the breakdown of hemoglobin

K

- **keratin** a fibrous protein found in hair, nails, and skin
- **keratitis** inflammation of the cornea
- **keratoconjunctivitis** inflammation of both the cornea and the conjunctiva
- **kidney** organ that filters the blood, producing urine
- **Kinyoun technique** a method of acid-fast staining that does not use heat to infuse the primary stain, carbolfuchsin, into acid-fast cells
- **Kirby-Bauer disk diffusion test** simple, rapid method for determining susceptibility and resistance of a bacterial pathogen to antibacterial drugs. The test involves drug-impregnated disks placed on an agar plate inoculated with a bacterial lawn.
- **Koplik's spots** white spots that form on the inner lining of the cheek of patients with measles
- **Krebs cycle** cyclic pathway during which each two-carbon unit entering the cycle is further oxidized, producing three NADH, one FADH₂, and one ATP by substrate-level phosphorylation, releasing two CO₂ molecules and regenerating the molecule used in the first step; also called the *citric acid cycle* or the *tricarboxylic acid cycle*
- **kuru** rare form of transmissible spongiform encephalopathy endemic to Papua New Guinea

L

- **lacrimal duct** connects the lacrimal gland to the lacrimal sac
- **lacrimal gland** a gland situated above the eye that secretes tears
- **lacrimal punctum** opening in each upper and lower eyelid
- **lacrimal sac** a to a reservoir for tears; also known as the dacrocyst or tear sac
- **lag period** the time between antigen exposure and production of antibodies
- **lag phase** interval before exponential growth of a microbial population during which cells adjust to a new environment
- **lagging strand** strand of DNA made discontinuously by DNA polymerase
- **laryngitis** inflammation of the larynx
- **laryngopharynx** lower portion of the pharynx that connects to the larynx
- **larynx** region of the respiratory tract containing the vocal cords; also referred to as the voice box
- **latent disease** disease that goes into a dormant nonreplicative state after the acute disease and can persist in this state for years, with the risk of reactivation back into acute disease
- **latent virus** virus that remains dormant in the host genome
- **lateral flow test** see immunochromatographic assays
- **leading strand** strand of DNA made continuously in the 5' to 3' direction by DNA polymerase
- **Legionnaires disease** atypical pneumonia occurring in older individuals; caused by the inhalation of *Legionella pneumophila* aerosolized in water
- **leishmaniasis** protozoan infection caused by *Leishmania* spp. and transmitted by sand flies
- **leprosy** see *Hansen's disease*
- **leptospirosis** bacterial infection of the kidney caused by *Leptospira* spp.; may spread to the liver, lungs, brain, and other organs
- **leukocidin** class of exotoxin that targets and lyses leukocytes
- **leukocytes** white blood cells of various types, including granulocytes, lymphocytes, and monocytes

- **leukotrienes** lipid-based chemical mediators produced by leukocytes and other tissue cells; promote inflammation and allergic responses
- **lichen** symbiotic association of a fungus with an algae or cyanobacterium
- **ligation** repair of the sugar-phosphate backbone of the DNA, making the DNA molecule continuous
- **light chains** the shorter identical peptide chains of an antibody molecule (two per antibody monomer), composed of variable and constant region segments
- **light-dependent reaction** process by which energy from sunlight is absorbed by pigment molecules in photosynthetic membranes and converted into stored chemical energy in the forms of ATP and NADPH
- **light-harvesting complex** group of multiple proteins and associated pigments that each may absorb light energy to become excited, and transfer this energy from one pigment molecule to another until the energy is delivered to a reaction center pigment
- **light-independent reaction** process by which chemical energy, in the form of ATP and NADPH produced by the light-dependent reactions, is used to fix inorganic CO₂ into organic sugar; usually referred to as the Calvin-Benson cycle
- **lincomycin** naturally produced protein synthesis inhibitor of the lincosamide class that binds to the 50S subunit, inhibiting peptide bond formation
- **lincosamides** class of protein synthesis inhibitors that are similar to macrolides
- **linked recognition** a mechanism whereby a B cell and the helper T cell with which it interacts recognize the same antigen
- **lipase** extracellular enzyme that degrades triglycerides
- **lipid bilayer** biological membranes composed of two layers of phospholipid molecules with the nonpolar tails associating to form a hydrophobic barrier between the polar heads; also called unit membrane
- **lipid** macromolecule composed primarily of carbon and hydrogen; source of nutrients for organisms, a storage form for carbon and energy, a part of the structure of membranes, and may function as hormones, pharmaceuticals, fragrances, and pigments

- **lipopolysaccharide (LPS)** lipid molecules with attached sugars that are found as components of gram-negative outer membranes
- **lipoprotein** conjugated protein attached to a lipid
- **listeriosis** bacterial disease caused from the ingestion of the microbe *Listeria monocytogenes*
- **lithotroph** chemotroph that uses inorganic chemicals as its electron source; also known as chemoautotroph
- **live attenuated vaccine** vaccine with live pathogen that has been attenuated to become less virulent in order to produce an active but subclinical infection
- **liver fluke** a trematode worm that affects the bile duct of the liver, including *Fasciola hepatica* and *F. gigantica*
- **local infection** infection in one limited area
- **log phase** interval of growth when cells divide exponentially; also known as the exponential growth phase
- **loiasis** a disease caused by the parasitic *Loa loa* worm, which is transmitted by deerflies; adult worms live in the subcutaneous tissue and cause inflammation, swelling, and eye pain as they migrate through the skin and the conjunctiva of the eye
- **lophotrichous** having a single tuft of flagella located at one end of a bacterial cell
- **low G+C gram-positive bacteria** bacteria that have less than 50% of guanine and cytosine nucleotides in their DNA
- **lumen** space inside the cisternae of the endoplasmic reticulum in eukaryotic cells
- **Lyme disease** tickborne disease caused by the spirochete *Borrelia burgdorferi*
- **lymph nodes** bean-shaped organs situated throughout the body that contain areas called germinal centers, which are rich in B and T lymphocytes; also contain macrophages and dendritic cells for antigen presentation
- **lymphadenitis** inflammation of the lymph nodes
- **lymphangitis** inflammation of the lymphatic vessels
- **lymphogranuloma venereum** infection caused by *Chlamydia trachomatis* in tropical regions
- **lyophilization** rapid freezing, followed by placement under a vacuum, of a material so that water is lost by sublimation, thereby inhibiting

microbial growth

- **lysis** destruction of the host cell
- **lysogen** bacterium carrying the prophage
- **lysogenic conversion (phage conversion)** alteration of host characteristics or phenotypes due to the presence of phage
- **lysogenic cycle** life cycle of some phages in which the genome of the infecting phage is integrated into the bacterial chromosome and replicated during bacterial reproduction until it excises and enters a lytic phase of the life cycle
- **lysogeny** process of integrating the phage into the host genome
- **lysosome** an organelle of the endomembrane system that contains digestive enzymes that break down engulfed material such as foodstuffs, infectious particles, or damaged cellular components
- **lytic cycle** infection process that leads to the lysis of host cells

M

- **M protein** a streptococcal cell wall protein that protects the bacteria from being phagocytized. It is associated with virulence and stimulates a strong immune response
- **macrolides** class of protein synthesis inhibitors containing a large, complex ring structure that binds to the 50S subunit, inhibiting peptide bond formation
- **macromolecule** polymer assembled from individual units, monomers, that bind together like building blocks
- **macronucleus** larger nucleus in ciliate protists that have two nuclei; polyploid with a reduced genome of metabolic genes and derived from the micronucleus
- **macronutrient** element required in abundance in cells; account for approximately 99% of the cell's dry weight
- **macrophages** monocytes that have left the bloodstream and differentiated into tissue-specific phagocytes
- **mad cow disease** form of transmissible spongiform encephalopathy primarily affecting cattle; can be transmitted to humans by consumption of contaminated cattle products

- **magnetosomes** inclusions in certain bacterial cells containing magnetic iron oxide or iron sulfide, which allows bacteria to align along a magnetic field by magnetotaxis
- **magnetotaxis** directional movement of bacterial cells using flagella in response to a magnetic field
- **magnification** the power of a microscope (or lens) to produce an image that appears larger than the actual specimen, expressed as a factor of the actual size
- **major histocompatibility complex (MHC)** collection of genes that code for MHC glycoproteins expressed on the surface of all nucleated cells
- **malaise** a general feeling of being unwell
- **malaria** potentially fatal, mosquito-borne protozoan infection caused by several species of *Plasmodium* and characterized by a relapsing fever, nausea, vomiting, and fatigue
- **mast cells** granulocytes similar in origin and function to basophils, but residing in tissues
- **matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF)** technique in which the sample (e.g., a microbe colony) is mixed with a special matrix and irradiated with a high-energy laser to generate characteristic gaseous ions that are subjected to mass spectral analysis, yielding mass spectra that may be compared to reference data for identification purposes
- **maturation** assembly of viral components to produce a functional virus
- **mature naïve T cell** a T cell that has exited the thymus after thymic selection but has not yet been activated
- **maximum growth pH** highest pH value that an organism can tolerate for growth
- **maximum growth temperature** highest temperature at which a microorganism will divide or survive
- **maximum permissible oxygen concentration** highest concentration of oxygen at which an organism will grow
- **measles** highly contagious respiratory disease caused by the measles virus (MeV); marked by an intense macular rash and high fever; also known as rubeola

- **mebendazole** antihelminthic drug of the benzimidazole class that binds to helminthic β -tubulin, preventing microtubule formation
- **mechanical transmission** transfer of a pathogen between hosts by a mechanical vector
- **mechanical vector** an animal that transfers a pathogen from one host to another or from a reservoir to a host without being infected by the pathogen itself
- **median infectious dose (ID₅₀)** concentration of pathogen that will produce active infection in 50% of test animals inoculated
- **median lethal dose (LD₅₀)** concentration of pathogen that kills 50% of infected test animals
- **medulla** loosely packed layer of fungal filaments located underneath the cortex of a lichen
- **membrane attack complex (MAC)** ring structure formed from complement proteins C6 through C9 that penetrates the membranes of a targeted cell, causing cell lysis and death
- **membrane filtration** method to remove bacteria from liquid, typically heat-sensitive solutions, using filters with an effective pore size of 0.2 μm or smaller, depending on need
- **membrane filtration technique** known volumes are vacuum filtered aseptically through a membrane with a pore size small enough to trap microorganisms, which are counted after growth on plates
- **membrane-bound ribosome** 80S eukaryotic ribosome attached to rough endoplasmic reticulum
- **membrane-disrupting toxin** toxin that affects cell membrane function by either forming pores or disrupting the phospholipid bilayer
- **memory B cell** an activated and differentiated B cell that is programmed to respond to secondary exposures to a specific antigen
- **memory helper T cell** a long-lived T cell programmed to recognize and quickly mount a secondary response to a specific pathogen upon re-exposure
- **memory** the ability of the specific adaptive immune system to quickly respond to pathogens to which it has previously been exposed
- **meninges** membranes that surround the brain
- **meningitis** inflammation of the meningeal membranes that surround the brain

- **meningococcal meningitis** bacterial infection caused by *Neisseria meningitidis* that results in an inflammation of the meninges
- **meningoencephalitis** inflammatory response that involves both the brain and the membranes that surround it
- **MERS** Middle East respiratory syndrome; first described in Saudi Arabia in 2013; caused by a zoonotic coronavirus that results in flu-like symptoms
- **mesophile** a microorganism that grows best at moderate temperatures, typically between about 20 °C and 45 °C
- **metabolism** all of the chemical reactions inside of cells
- **metachromatic granule** a type of inclusion containing volutin, a polymerized inorganic phosphate that appears red when stained with methylene blue
- **metagenomics** the sequencing of genomic fragments from microbial communities, allowing researchers to study genes from a collection of multiple species
- **metatranscriptomics** the science of studying a collection of mRNA molecules produced from microbial communities; involves studying gene expression patterns from a collection of multiple species
- **methanogen** microorganism that produces gaseous methane
- **methicillin-resistant *Staphylococcus aureus* (MRSA)** pathogen resistant to all β -lactams through acquisition of a new low-affinity penicillin-binding protein, and often resistant to many other drug classes
- **metronidazole** antibacterial and antiprotozoan drug of the nitroimidazole class that is activated in anaerobic target cell and introduces DNA strand breakage, thus interfering with DNA replication in target cells
- **MHC I molecule** glycoprotein expressed on the surface of all nucleated cells and involved in the presentation of normal “self” antigens and foreign antigens from intracellular pathogens
- **MHC II molecule** glycoprotein expressed only on the surface of antigen-presenting cells and involved in the presentation of foreign antigens from pathogens ingested by phagocytosis
- **micelle** simple spherical arrangement of amphipathic lipid molecules with nonpolar tails aggregated within the interior and polar heads forming the outer surface

- **microaerophile** organism that requires oxygen at levels lower than atmospheric concentration
- **microarray analysis** a technique used to compare two samples of genomic DNA or cDNA; the DNA or cDNA fragments are immobilized on a chip and labeled with different fluorescent dyes, allowing for comparison of sequences or gene-expression patterns
- **microbe** generally, an organism that is too small to be seen without a microscope; also known as a microorganism
- **microbial death curve** graphical representation of the progress of a particular microbial control protocol
- **microbial ecology** study of the interactions between microbial populations microbiology the study of microorganisms
- **microbiome** all prokaryotic and eukaryotic microorganisms that are associated with a certain organism
- **microfilament** cytoskeletal fiber composed of actin filaments
- **microinjection** the direct injection of DNA into the cytoplasm of a eukaryotic cell using a glass micropipette
- **micronucleus** smaller nucleus in ciliate protists that have two nuclei; diploid, somatic, and used for sexual reproduction through conjugation
- **micronutrient** indispensable element present in cells in lower amounts than macronutrients; also called *trace element*
- **microorganism** generally, an organism that is too small to be seen without a microscope; also known as a microbe
- **microsporidia** fungi that lack mitochondria, centrioles, and peroxisomes; some can be human pathogens
- **microtiter plates** plastic dishes with multiple small wells
- **microtubule** hollow tube composed of tubulin dimers (α and β tubulin); the structural component of the cytoskeleton, centrioles, flagella, and cilia
- **miliary tuberculosis** hematogenous dissemination and spread of *Mycobacterium tuberculosis* from tubercles
- **minimal bactericidal concentration (MBC)** lowest antibacterial drug concentration that kills $\geq 99.9\%$ of a starting inoculum of bacteria
- **minimal inhibitory concentration (MIC)** lowest concentration of an antibacterial drug that inhibits visible growth of a bacterial strain
- **minimum growth pH** lowest pH value that an organism can tolerate for growth

- **minimum growth temperature** lowest temperature at which a microorganism will divide or survive
- **minimum permissible oxygen concentration** lowest concentration of oxygen at which an organism will grow
- **missense mutation** point mutation that results in a different amino acid being incorporated into the resulting polypeptide
- **mitochondrial matrix** the innermost space of the mitochondrion enclosed by two membranes; the location of many metabolic enzymes as well as the mitochondrial DNA and 70S ribosomes
- **mitochondrion (plural: mitochondria)** large, complex organelle that is the site of cellular respiration in eukaryotic cells
- **mode of action** way in which a drug affects a microbe at the cellular level
- **moist-heat sterilization** protocol that involves steam under pressure in an autoclave, allowing the steam to reach temperatures higher than the boiling point of water
- **mold** a multicellular fungus, typically made up of long filaments
- **molecular cloning** the purposeful fragmentation of DNA followed by attachment to another piece of DNA to produce a recombinant molecule, followed by introduction of this recombinant molecule into an easily manipulated host to allow for the creation of multiple copies of a gene of interest
- **monoclonal antibodies (mAbs)** antibodies produced *in vitro* that only bind to a single epitope
- **monocular** having a single eyepiece
- **monocytes** large, agranular, mononuclear leukocytes found in the peripheral blood; responsible for phagocytosis of pathogens and damaged cells
- **monoecious** refers to sexually reproducing organisms in which individuals have both male and female reproductive organs
- **monomer** small organic molecule that binds with like molecules, forming a polymer or macromolecule
- **monosaccharide** monomer for the synthesis of carbohydrate polymers; the simplest carbohydrate, called a *simple sugar*
- **monotrichous** having one flagellum, typically located on one end of the bacterial cell
- **morbidity** a state of illness

- **Morbidity and Mortality Weekly Report (MMWR)** the trade/industry publication for epidemiologists, reporting US public health data compiled by the CDC
- **morbidity rate** the number of cases of a disease expressed as a percentage of the population or number per standard part of the population, such as 100,000
- **mordant** a chemical added to a specimen that sets a stain
- **mortality** death
- **mortality rate** the number of deaths from a disease expressed as a percentage of the population or number per standard part of the population, such as 100,000
- **most probable number (MPN)** statistical value representing the viable bacterial population in a sample obtained after a series of dilutions and multiple tube inoculations
- **mRNA** short-lived type of RNA that serves as the intermediary between DNA and the synthesis of protein products
- **mucociliary escalator** system by which mucus and debris are propelled up and out of the respiratory tract by the beating of respiratory cilia and the mechanical actions of coughing or swallowing
- **mucormycosis** rare form of pneumonia that can be caused by an invasive infection of different fungi in the order Mucorales, such as *Rhizopus* or *Mucor*
- **mucous membrane** moist layer of epithelial cells and interspersed goblet cells that lines the inner surfaces of the body, usually bathed in antimicrobial secretions from the cells of the membrane
- **mucus** viscous secretion produced by cells and glands in various mucous membranes throughout the body; helps trap and remove microbes and debris from the body
- **multidrug-resistant microbes (MDR)** group of pathogens that carry one or more resistance mechanisms, making them resistant to multiple antimicrobials; also called superbugs
- **multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB)** strains of *M. tuberculosis* that are resistant to both rifampin and isoniazid, the drug combination typically prescribed for the treatment of tuberculosis
- **multiple sclerosis** autoimmune attack on the myelin sheaths and nerve cells in the central nervous system

- **mumps** a viral illness that causes swelling of the parotid glands; rare in the United States because of effective vaccination
- **murine typhus** fleaborne infection caused by *Rickettsia typhi* and characterized by fever, rash, and pneumonia
- **mutagen** type of chemical agent or radiation that can induce mutations
- **mutant** organism harboring a mutation that often has a recognizable change in phenotype compared to the wild type
- **mutation** heritable change in the DNA sequence of an organism
- **mutualism** type of symbiosis in which two populations benefit from, and depend on, each other
- **myasthenia gravis** autoimmune disease affecting the acetylcholine receptors in the neuromuscular junction, resulting in weakened muscle contraction capability
- **mycelium** vegetative network of branched, tubular hyphae
- **mycolic acids** waxy molecules associated with peptidoglycan in some gram-positive, acid-fast bacteria, chiefly mycobacteria
- **mycology** the study of fungi
- ***Mycoplasma pneumonia*** also known as walking pneumonia; a milder form of atypical pneumonia caused by *Mycoplasma pneumoniae*
- **mycoses (mycosis, sing.)** refers to diseases caused by fungi
- **mycotoxin** biologically active product of pathogenic fungi that causes adverse changes in the host cells
- **myelin sheath** insulating layer that surrounds the axon of some neurons and helps to promote signal propagation
- **myocarditis** inflammation of the heart muscle tissues

N

- **naïve mature B cell** a B cell that has not yet been activated
- **naked virus** virus composed of a nucleic acid core, either DNA or RNA, surrounded by a capsid
- **nalidixic acid** member of the quinolone family that functions by inhibiting the activity of DNA gyrase, blocking DNA replication
- **narrow-spectrum antimicrobial** drug that targets only a specific subset of microbes
- **nasal cavity** air-filled space in the skull immediately behind the nose

- **nasolacrimal duct** tear duct connecting the lacrimal glands to the nasal cavity
- **nasolacrimal duct** tear fluid flows from each eye through this duct to the inner nose
- **nasopharynx** part of the upper throat (pharynx) extending from the posterior nasal cavity; carries air inhaled through the nose
- **native structure** three-dimensional structure of folded fully functional proteins
- **natural active immunity** immunity that develops as a result of natural infection with a pathogen
- **natural antibiotic** antimicrobial compound that is produced naturally by microorganisms in nature
- **natural killer cells (NK cells)** lymphoid cells that recognize and destroy abnormal target cells by inducing apoptosis
- **natural passive immunity** transfer of maternal antibodies from mother to fetus (transplacentally) or infant (via breastmilk)
- **necrotizing fasciitis** a serious infection, also known as flesh-eating disease, that leads to rapid destruction of tissue through the action of exotoxin A; it can be caused by *S. pyogenes* or several other bacterial species
- **negative (–) single-strand RNA (–ssRNA)** a viral RNA strand that cannot be translated until it is replicated into positive single-strand RNA by viral RNA-dependent RNA polymerase
- **negative stain** a stain that produces color around the structure of interest while not coloring the structure itself
- **Nematoda** phylum comprising roundworms
- **neonatal herpes** herpes infection of the newborn, generally caused by infection during birth
- **neonatal meningitis** meningitis caused by Group B streptococcus and occurring primarily in neonates (less than 2 months old)
- **neonatal tetanus** tetanus acquired through infection of the cut umbilical cord
- **neurocysticercosis** parasitic invasion of brain tissues by the larvae of the pork tapeworm, *Taenia solium*
- **neuromycosis** any fungal infection of the nervous system
- **neuron** specialized cell found throughout the nervous system that transmits signals through the nervous system using electrochemical

processes

- **neuropathy** numbness or tingling sensation caused by damage to peripheral nerves
- **neurotoxoplasmosis** disease caused by the invasion of brain tissues by the protozoan *Toxoplasma gondii*; typically only affects immunocompromised patients
- **neurotransmitter** compound that is released at the synapse of neurons to stimulate or suppress the actions of other cells
- **neutralism** type of symbiosis that does not affect either of the two populations
- **neutralization** binding of an antibody to a pathogen or toxin, preventing attachment to target cells
- **neutrophile** organism that grows best at a near a neutral pH of 6.5–7.5
- **neutrophils** leukocytes with a multilobed nucleus found in large numbers in peripheral blood; able to leave the bloodstream to phagocytose pathogens in infected tissues; also called polymorphonuclear neutrophils (PMNs)
- **next generation sequencing** a group of automated techniques used for rapid DNA sequencing
- **nicotine adenine dinucleotide (NAD⁺/NADH)** oxidized/reduced forms of an electron carrier in cells
- **nicotine adenine dinucleotide phosphate (NADP⁺/NADPH)** oxidized/reduced forms of an electron carrier in cells
- **nitrogen fixation** bacterial biochemical pathways that incorporate inorganic nitrogen gas into organic forms more easily used by other organisms
- **nitrogenous base** nitrogen-containing ring structure within a nucleotide that is responsible for complementary base pairing between nucleic acid strands
- **noncoding DNA** regions of an organism's genome that, unlike genes, do not encode proteins
- **noncommunicable disease** disease that is not transmitted from one person to another
- **noncompetitive (allosteric) inhibitor** molecule that binds to allosteric sites, inducing a conformational change in the enzyme's structure that prevents it from functioning

- **noncritical item** object that may contact intact skin but does not penetrate it; requires cleanliness but not a high level of disinfection
- **noncyclic photophosphorylation** pathway used in photosynthetic organisms when both ATP and NADPH are required by the cell
- **nonenveloped virus** naked virus
- **nongonococcal urethritis (NGU)** a nonspecific infection of the urethra that is not caused by *Neisseria gonorrhoeae*
- **noninfectious disease** disease caused by something other than an infectious agent (e.g., genetics, environment, nutritional deficiencies)
- **nonionizing radiation** low-energy radiation, like ultraviolet light, that can induce dimer formation between two adjacent pyrimidine bases, resulting in DNA polymerase stalling and possible formation of a frameshift mutation
- **nonsense mutation** point mutation that converts a codon encoding an amino acid (a sense codon) into a stop codon (a nonsense codon)
- **nontreponemal serologic tests** qualitative and quantitative indirect diagnostic tests for syphilis
- **northern blot** a technique in molecular genetics used to detect the amount of RNA made by gene expression within a tissue or organism sample; RNA fragments within a sample are separated by agarose gel electrophoresis, immobilized on a membrane, and then exposed to a specific DNA probe labeled with a radioactive or fluorescent molecular beacon to aid in detection
- **nosocomial disease** disease acquired in a hospital setting
- **notifiable disease** a disease for which all cases must legally be reported to regional, state, and/or federal public health agencies
- **nuclear envelope** (also called the nuclear membrane) a structure defining the boundary of the nucleus; composed of two distinct lipid bilayers that are contiguous with each other and with the endoplasmic reticulum
- **nuclear lamina** a meshwork of intermediate filaments (mainly lamins) found just inside the nuclear envelope; provides structural support to the nucleus
- **nucleic acid** class of macromolecules composed of nucleotide monomers polymerized into strands
- **nucleoid** concentrated area of DNA genome and associated proteins found in a prokaryotic cell that is not surrounded by a membrane

- **nucleoid-associated protein (NAP)** protein that assists in the organization and packaging of the chromosome in prokaryotic cells
- **nucleolus** a dense region within the nucleus where ribosomal RNA biosynthesis occurs and preribosomal complexes are made
- **nucleoside analog** chemical that is structurally similar to a normal nucleotide base that can be incorporated into DNA instead of normal bases during replication but that has different base pairing rules than the normal base for which it was substituted, inducing mutation
- **nucleotide excision repair (dark repair)** enzymatic mechanism to repair pyrimidine dimers by cutting the dimer-containing DNA strand on both sides of dimer, removing the intervening strand and replacing the bases with the correct ones
- **nucleotide** nucleic acid monomer composed of a pentose sugar, a phosphate group, and a nitrogenous base
- **nucleus** a membrane-bound structure of eukaryotic cells that houses the DNA genome
- **numerical aperture** a measure of a lens's ability to gather light

O

- **objective lenses** on a light microscope, the lenses closest to the specimen, typically located at the ends of turrets
- **obligate aerobe** organism that requires oxygen for growth
- **obligate anaerobe** organism that dies in the presence of oxygen
- **obligate intracellular pathogen** microorganism that cannot synthesize its own ATP and, therefore, must rely on a host cell for energy; behaves like a parasite when inside a host cell, but is metabolically inactive outside of a host cell
- **observational study** a type of scientific study that involves measurement of study subjects on variables hypothesized to be associated with the outcome of interest, but without any manipulation of the subjects
- **ocular lens** on a microscope, the lens closest to the eye (also called an eyepiece)
- **oil immersion lens** a special objective lens on a microscope designed to be used with immersion oil to improve resolution

- **Okazaki fragment** short fragment of DNA made during lagging strand synthesis
- **oligopeptide** peptide having up to approximately 20 amino acids
- **oligotroph** organism capable of living in low-nutrient environments
- **opacity** the property of absorbing or blocking light
- **operator** DNA sequence located between the promoter region and the first coding gene to which a repressor protein can bind
- **operon** a group of genes with related functions often found clustered together within the prokaryotic chromosome and transcribed under the control of a single promoter and operator repression sequence
- **ophthalmia neonatorum** inflammation of the conjunctiva in newborns caused by *Neisseria gonorrhoeae* transmitted during childbirth
- **opisthotonos** characteristic symptom of tetanus that results in uncontrolled muscular spasms and backward arching of the neck and spine
- **opportunistic pathogen** microorganism that can cause disease in individuals with compromised host defenses
- **opsonin** any molecule that binds to and coats the outside of a pathogen, identifying it for destruction by phagocytes (examples include antibodies and the complement proteins C3b and C4b)
- **opsonization** process of coating a pathogen with a chemical substance (an opsonin) that allows phagocytic cells to recognize, engulf, and destroy the pathogen more easily
- **optimum growth pH** the pH at which an organism grows best
- **optimum growth temperature** the temperature at which a microorganism's growth rate is highest
- **optimum oxygen concentration** the ideal concentration of oxygen for a particular microorganism
- **oral herpes** an infection caused by herpes simplex virus that results in cold sores, most commonly on and around the lips
- **oral thrush** *Candida* infection of the mouth
- **orchitis** inflammation of one or both of the testes
- **organic molecule** composed primarily of carbon; typically contains at least one carbon atom bound to one or more hydrogen atoms
- **organotroph** chemotroph that uses organic molecules as its electron source; also known as chemoheterotroph

- **origin of replication** specific nucleotide sequence where replication begins
- **oropharynx** area where air entering mouth enters the pharynx
- **osmosis** diffusion of water across a semipermeable membrane
- **osmotic pressure** the force or pressure generated by water diffusing across a semipermeable membrane, driven by differences in solute concentration across the membrane
- **osteomyelitis** inflammation of bone tissue
- **otitis externa** an infection of the external ear canal, most commonly caused by *Pseudomonas aeruginosa*; often called swimmer's ear
- **otitis** inflammation of the ear
- **otitis media with effusion** accumulation of fluid inside the middle ear with or without infection
- **Ouchterlony assay** test in which antigen and antisera are added to neighboring wells in an agar gel, allowing visualization of precipitin arcs
- **outer membrane** a phospholipid bilayer external to the peptidoglycan layer found in gram-negative cell walls
- **oxazolidinones** class of synthetic protein synthesis inhibitors that interfere with formation of the initiation complex for translation and prevent translocation of the growing protein from the ribosomal A site to the P site
- **oxidation reaction** chemical reaction that removes electrons (often as part of H atoms) from donor molecules, leaving them oxidized
- **oxidative phosphorylation** mechanism for making ATP that uses the potential energy stored within an electrochemical gradient to add P_i to ADP
- **oxygenic photosynthesis** type of photosynthesis found in plants, algae, and cyanobacteria, and in which H_2O is used as the electron donor to replace an electron lost by a reaction center pigment, resulting in oxygen as a byproduct

P

- **P (peptidyl) site** functional site of an intact ribosome that binds charged tRNAs carrying amino acids that have formed peptide bonds

with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA

- **palatine tonsil** lymphoid tissue located near the oropharynx
- **pandemic disease** an epidemic that is worldwide as opposed to regional
- **papilloma** growth on the skin associated with infection by any of the human papilloma viruses (HPV); commonly known as a wart
- **paracrine function** refers to a cytokine signal released from a cell to a receptor on a nearby cell
- **parasitism** type of symbiosis in which one population benefits while harming the other parasitology the study of parasites
- **parenteral route** means of entry by a pathogen through skin or mucous membranes when these barriers are breached
- **paroxysmal stage** most serious stage of pertussis (whooping cough), characterized by severe and prolonged coughing spells
- **passive carrier** an individual capable of transmitting a pathogen to another individual without becoming infected
- **passive immunity** adaptive immune defenses received from another individual or animal
- **pasteurization** form of microbial control using heat that is applied to foods; kills pathogens and reduces the number of spoilage-causing microbes while maintaining food quality
- **pathogen** a disease-causing microorganism
- **pathogen-associated molecular patterns (PAMPs)** common molecular motifs found on pathogens
- **pathogenicity** ability of a microbial agent to cause disease
- **pattern recognition receptors (PRRs)** receptors on the surface or in the interior of phagocytic cells that bind to pathogen-associated molecular patterns (PAMPs)
- **pellicle** structure that underlies the plasma membrane in protists, providing additional support
- **pelvic inflammatory disease (PID)** infection of the female reproductive organs that may spread from the vagina to the cervix, uterus, fallopian tubes, and ovaries
- **penetration** entry of phage or virus into a host cell through injection, endocytosis, or membrane fusion

- **penicillin** β -lactam antibacterial that was the first cell wall synthesis inhibitor developed
- **penis** external genital organ in males through which urine and semen are discharged
- **pentamidine** antiprotozoan drug that appears to degrade kDNA in target cells, as well as inhibit protein synthesis
- **pentose phosphate pathway (PPP)** alternative glycolytic pathway that produces intermediates used for the biosynthesis of nucleotides and amino acids; also called the *phosphogluconate pathway* or the *hexose monophosphate shunt*
- **peptic ulcer** an ulcer in the lining of the stomach or duodenum, often associated with *Helicobacter pylori*
- **peptide bond** bond between the carboxyl group of one amino acid and the amine group of another; formed with the loss of a water molecule
- **peptidoglycan (murein)** the polymer of alternating N-acetylmuramic acid NAM and N-acetylglucosamine (NAG) subunits linked together by peptide chains; a major constituent of bacterial cell walls
- **peptidyl transferase** RNA-based ribozyme that is part of the 50S ribosomal subunit and catalyzes formation of the peptide bond between the amino acid bound to a tRNA and the growing polypeptide chain
- **perforin** compound released from a natural killer cell that creates pores in the target cell through which other toxins (particularly granzymes) can gain access to the cytoplasm
- **pericarditis** inflammation of the sac that surrounds the heart
- **period of convalescence** fifth stage of acute disease, during which the patient returns to normal function
- **period of decline** fourth stage of disease, during which the number of pathogens present in the host decreases, along with signs and symptoms of disease
- **period of illness** third stage of acute disease, during which the number of pathogens present in the host is greatest and the signs and symptoms of disease are most severe
- **periodontal disease** a condition in which the gums are inflamed and may erode
- **periodontitis** inflammation of the gums that is more severe than gingivitis, spreading deeper into the tissues

- **peripheral nervous system** network of neurons that connects the CNS with organs, sensory organs, and muscles throughout the body
- **peripheral tolerance** mechanism by which regulatory T cells inhibit self-reactive immune responses in T cells that have already exited the thymus
- **periplasmic space** the space between the cell wall and the plasma membrane, primarily in gram-negative bacteria
- **peristalsis** muscular contractions of the gastrointestinal tract that propel ingested material through the stomach, intestines, and, eventually, through the rectum and out of the body
- **peritrichous** having numerous flagella covering the entire surface of a bacterial cell
- **peroxidase** enzyme that catalyzes the detoxification of peroxides
- **peroxisome** in eukaryotic cells, a membrane-bound organelle (not part of the endomembrane system) that produces hydrogen peroxide to break down various types of molecules; also plays a role in lipid biosynthesis
- **peroxygen** type of strong oxidizing agent that causes free radical formation in cells; can be used as a disinfectant or antiseptic
- **persister** dormant cell that survives in the death phase and is resistant to most antibiotics
- **pertussis** contagious illness caused by *Bordetella pertussis* that causes severe coughing fits followed by a whooping sound during inhalation; commonly known as whooping cough
- **pertussis toxin** main virulence factor accounting for the symptoms of whooping cough
- **petechiae** small red or purple spots on the skin that result from blood leaking out of damaged vessels
- **Petroff-Hausser counting chamber** calibrated slide that allows counting of bacteria in a specific volume under a microscope
- **Peyer's patches** lymphoid tissue in the ileum that monitors and fights infections
- **phagemid** a plasmid capable of being replicated as a plasmid and also incorporated into a phage head
- **phagocytosis** a type of endocytosis in which large particles are engulfed by membrane invagination, after which the particles are

enclosed in a pocket, which is pinched off from the membrane to form a vacuole

- **phagolysosome** compartment in a phagocytic cell that results when the phagosome is fused with the lysosome, leading to the destruction of the pathogens inside
- **phagosome** compartment in the cytoplasm of a phagocytic cell that contains the phagocytosed pathogen enclosed by part of the cell membrane
- **pharmacogenomics (toxicogenomics)** the evaluation of the effectiveness and safety of drugs on the basis of information from an individual's genomic sequence as well as examination of changes in gene expression in response to the drug
- **pharyngitis** inflammation of the pharynx
- **pharynx** region connecting the nose and mouth to the larynx: the throat
- **phase-contrast microscope** a light microscope that uses an annular stop and annular plate to increase contrast
- **phenol coefficient** measure of the effectiveness of a chemical agent through comparison with that of phenol on *Staphylococcus aureus* and *Salmonella enterica* serovar Typhi
- **phenolics** class of chemical disinfectants and antiseptics characterized by a phenol group that denatures proteins and disrupts membranes
- **phenotype** observable characteristics of a cell or organism
- **phosphodiester bonds** linkage whereby the phosphate group attached to the 5' carbon of the sugar of one nucleotide bonds to the hydroxyl group of the 3' carbon of the sugar of the next nucleotide
- **phosphogluconate pathway** see *pentose phosphate pathway*
- **phospholipase** enzyme that degrades phospholipids
- **phospholipid** complex lipid that contains a phosphate group
- **phospholipid-derived fatty acids (PLFA) analysis** technique in which membrane phospholipids are saponified to release the fatty acids of the phospholipids, which can be subjected to FAME analysis for identification purposes
- **phosphorescence** the ability of certain materials to absorb energy and then release that energy as light after a delay
- **photosynthesis** process whereby phototrophic organisms convert solar energy into chemical energy that can then be used to build

carbohydrates

- **photosynthetic pigment** pigment molecule used by a cell to absorb solar energy; each one appears the color of light that it transmits or reflects
- **photosystem** organized unit of pigments found within a photosynthetic membrane, containing both a light-harvesting complex and a reaction center
- **phototaxis** directional movement using flagella in response to light
- **phototroph** organism that gets its energy from light
- **phototrophic bacteria** nontaxonomic group of bacteria that use sunlight as their primary source of energy
- **phylogeny** the evolutionary history of a group of organisms
- **phytoplankton** photosynthetic plankton
- **pia mater** fragile and innermost membrane layer surrounding the brain
- **pili** long protein extensions on the surface of some bacterial cells; specialized F or sex pilus aids in DNA transfer between cells
- **pinocytosis** a type of endocytosis in which small dissolved materials are endocytosed into smaller vesicles
- **plague** infectious epidemic disease caused by *Yersinia pestis*
- **plankton** microscopic organisms that float in the water and are carried by currents; they may be autotrophic (phytoplankton) or heterotrophic (zooplankton)
- **planktonic** free-floating or drifting in suspension
- **plantibodies** monoclonal antibodies produced in plants that are genetically engineered to express mouse or human antibodies
- **plaque** clear area on bacterial lawn caused by viral lysis of host cells
- **plasma cell** activated and differentiated B cell that produces and secretes antibodies
- **plasma** fluid portion of the blood that contains all clotting factors
- **plasma membrane** (also called the cell membrane or cytoplasmic membrane) lipid bilayer with embedded proteins that defines the boundary of the cell
- **plasmalemma** protist plasma membrane
- **plasmid** small, circular, double-stranded DNA molecule that is typically independent from the bacterial chromosome
- **plasmolysis** the separation of the plasma membrane away from the cell wall when a cell is exposed to a hypertonic environment

- **platelets** cell fragments in the peripheral blood that originate from megakaryocyte cells in the bone marrow; also called thrombocytes
- **Platyhelminthes** phylum comprising flatworms
- **pleconaril** an antiviral drug targeting picornaviruses that prevents the uncoating of virus particles upon their infection of host cells
- **pleomorphic** able to change shape
- **pneumococcal meningitis** bacterial infection caused by *Streptococcus pneumoniae* that results in an inflammation of the meninges
- ***Pneumocystis pneumonia*** common pulmonary infection in patients with AIDS; caused by *P. jirovecii*
- **pneumonia** pulmonary inflammation that causes the lungs to fill with fluids
- **pneumonic plague** rare form of plague that causes massive hemorrhages in the lungs and is communicable through aerosols
- **point mutation** mutation, most commonly a base substitution, that affects a single base pair
- **point source spread** a form of common source spread in which the transmission of a disease from the source occurs for a brief period that is less than the pathogen's incubation period
- **polar tubule** a tube-like structure produced by spores of parasitic Microsporidia fungi that pierces host cell membranes
- **poliomyelitis (polio)** disease caused by an infection of the enteric polio virus characterized by inflammation of the motor neurons of the brain stem and spinal cord; can result in paralysis
- **poly-A tail** string of approximately 200 adenine nucleotides added to the 3' end of a eukaryotic primary mRNA transcript to stabilize it
- **polyacrylamide gel electrophoresis (PAGE)** a method for separating populations of proteins and DNA fragments during Sanger sequencing of varying sizes by differential migration rates caused by a voltage gradient through a vertical gel matrix
- **polycistronic mRNA** single mRNA molecule commonly produced during prokaryotic transcription that carries information encoding multiple polypeptides
- **polyclonal antibodies** antibodies produced in a normal immune response, in which multiple clones of B cells respond to many different epitopes on an antigen

- **polyenes** class of antifungal drugs that bind to ergosterol to form membrane pores, disrupting fungal cell membrane integrity
- **polyhedral virus** virus with a three-dimensional shape with many facets
- **polyhydroxybutyrate (PHB)** a type of cellular inclusion surrounded by a phospholipid monolayer embedded with protein
- **polylinker site** or **multiple cloning site (MCS)** a short sequence containing multiple unique restriction enzyme recognition sites that are used for inserting foreign DNA into the plasmid after restriction digestion of both the foreign DNA and the plasmid
- **polymer** macromolecule composed of individual units, monomers, that bind together like building blocks.
- **polymerase chain reaction (PCR)** an *in vitro* molecular technique that rapidly amplifies the number of copies of specific DNA sequences to make the amplified DNA available for other analyses
- **polymorphonuclear neutrophil (PMN)** see *neutrophils*
- **polymyxins** lipophilic polypeptide antibiotics that target the lipopolysaccharide component of gram-negative bacteria and ultimately disrupt the integrity of their outer and inner membranes
- **polypeptide** polymer having from approximately 20 to 50 amino acids
- **polyphyletic** refers to a grouping of organisms that is not descended from a single common ancestor
- **polyribosome (polysome)** structure including an mRNA molecule that is being translated by multiple ribosomes concurrently
- **polysaccharide** polymer composed of hundreds of monosaccharides linked together by glycosidic bonds; also called *glycans*
- **portal of entry** anatomical feature of the body through which pathogens can enter host tissue
- **portal of exit** anatomical feature of the body through which pathogens can leave diseased individual
- **positive (+) strand** viral RNA strand that acts like messenger RNA and can be directly translated inside the host cell
- **positive stain** a stain that colors the structure of interest
- **pour plate method** a technique used for inoculating plates with diluted bacterial samples for the purpose of cell counting; cells are mixed with warm liquid agar before being poured into Petri dishes

- **praziquantel** antihelminthic drug that induces a calcium influx into tapeworms, leading to spasm and paralysis
- **precipitin** complex lattice of antibody and antigen that becomes too large to stay in solution
- **precipitin ring test** assay in which layers of antisera and antigen in a test tube form precipitin at the interface of the two solutions
- **prevalence** the total number or proportion of individuals in a population ill with a specific disease
- **primary amoebic meningoencephalitis (PAM)** acute and deadly parasitic infection of brain tissues by the amoeba *Naegleria fowleri*
- **primary antibody** in a sandwich ELISA, the antibody that is attached to wells of a microtiter plate to capture antigen from a solution, or in an indirect ELISA, the antigen-specific antibody present in a patient's serum
- **primary cell culture** cells taken directly from an animal or plant and cultured in vitro
- **primary immunodeficiency** genetic condition that results in impaired immune function
- **primary infection** initial infection produced by a pathogen
- **primary lymphoid tissue** one of two types of lymphatic tissue; comprises bone marrow and the thymus
- **primary pathogen** microorganism that can cause disease in the host regardless of the effectiveness of the host's immune system
- **primary response** the adaptive immune response produced upon first exposure to a specific antigen
- **primary stain** refers, in differential staining techniques, to the first dye added to the specimen
- **primary structure** bonding sequence of amino acids in a polypeptide chain **protein** macromolecule that results when the number of amino acids linked together becomes very large, or when multiple polypeptides are used as building subunits
- **primary transcript** RNA molecule directly synthesized by RNA polymerase in eukaryotes before undergoing the additional processing required to become a mature mRNA molecule
- **primase** RNA polymerase enzyme that synthesizes the RNA primer required to initiate DNA synthesis

- **primer** short complementary sequence of five to 10 RNA nucleotides synthesized on the template strand by primase that provides a free 3'-OH group to which DNA polymerase can add DNA nucleotides
- **prion** acellular infectious particle consisting of just proteins that can cause progressive diseases in animals and humans
- **prodromal period** second stage of acute disease, during which the pathogen continues to multiply in the host and nonspecific signs and symptoms become observable
- **progeny virus** newly assembled virions ready for release outside the cell
- **proglottid** body segment of a cestode (tapeworm)
- **prokaryote** an organism whose cell structure does not include a membrane-bound nucleus
- **prokaryotic cell** a cell lacking a nucleus bound by a complex nuclear membrane
- **promoter** DNA sequence onto which the transcription machinery binds to initiate transcription
- **propagated spread** the progression of an infectious disease from person to person, either indirectly or directly, through a population of susceptible individuals as one infected individual transmits the agent to others, who transmit it to others yet again
- **prophage** phage genome that has incorporated into the host genome
- **prospective study** a research design that follows cases from the beginning of the study through time to associate measured variables with outcomes
- **prostate gland** gland that contributes fluid to semen
- **prostatitis** inflammation of the prostate gland
- **protease** enzyme involved in protein catabolism that removes individual amino acids from the ends of peptide chains
- **protease inhibitor** class of antiviral drugs, used in HIV therapy and hepatitis C therapy, that inhibits viral-specific proteases, preventing viral maturation
- **protein signature** an array of proteins expressed by a cell or tissue under a specific condition
- **Proteobacteria** phylum of gram-negative bacteria
- **proteomic analysis** study of all accumulated proteins of an organism

- **proteomics** the study of the entire complement of proteins in an organism; involves monitoring differences in gene expression patterns between cells at the protein level
- **protists** informal name for diverse group of eukaryotic organisms, including unicellular, colonial, and multicellular types that lack specialized tissues
- **proton motive force** electrochemical gradient formed by the accumulation of hydrogen ions (also known as protons) on one side of a membrane relative to the other protozoan (plural: protozoa) a unicellular eukaryotic organism, usually motile
- **protozoans** informal term for some protists, generally those that are nonphotosynthetic, unicellular, and motile protozoology the study of protozoa
- **provirus** animal virus genome that has integrated into the host chromosome
- **pseudohyphae** short chains of yeast cells stuck together
- **pseudomembrane** grayish layer of dead cells, pus, fibrin, red blood cells, and bacteria that forms on mucous membranes of the nasal cavity, tonsils, pharynx, and larynx of individuals with diphtheria
- **pseudomembranous colitis** inflammation of the large intestine with the formation of a pseudomembrane; caused by *C. difficile*
- **pseudopodia** temporary projections involved in amoeboid movement; these “false feet” form by gel-sol cycling of actin polymerization/depolymerization
- **psittacosis** zoonotic *Chlamydothyla* infection from birds that causes a rare form of pneumonia
- **psoriasis** autoimmune disease involving inflammatory reactions in and thickening of skin
- **psychrophile** a microorganism that grows best at cold temperatures; most have an optimum growth temperature of about 15 °C and can survive temperatures below 0 °C; most cannot survive temperatures above 20 °C
- **psychrotroph** a microorganism that grows best at cool temperatures, typically between about 4 °C and 25 °C, with optimum growth at about 20 °C
- **puerperal sepsis** sepsis associated with a bacterial infection incurred by a woman during or after childbirth

- **purines** nitrogenous bases containing a double-ring structure with a six-carbon ring fused to a five-carbon ring; includes adenine and guanine
- **purple nonsulfur bacteria** phototrophic bacteria that are similar to purple sulfur bacteria except they use hydrogen rather than hydrogen sulfide for oxidation
- **purple sulfur bacteria** phototrophic bacteria that oxidize hydrogen sulfide into elemental sulfur and sulfuric acid; their purple color is due to the pigments bacteriochlorophylls and carotenoids
- **purulent** an infection that produces pus; suppurative
- **pus** accumulation of dead pathogens, neutrophils, tissue fluid, and other bystander cells that may have been killed by phagocytes at the site of an infection
- **pyelonephritis** an infection of one or both kidneys
- **pyocyanin** blue pigments produced by some strains of *Pseudomonas aeruginosa*
- **pyoderma** any suppurative (pus-producing) infection of the skin
- **pyoverdinin** a water-soluble, yellow-green or yellow-brown pigment produced by some strains of *Pseudomonas aeruginosa*
- **pyrimidines** nitrogenous bases containing a single six-carbon ring; includes cytosine and thymine in DNA
- **pyrophosphate (PPi)** two connected phosphate groups in solution
- **pyuria** pus or white blood cells in the urine

Q

- **Q fever** highly infectious zoonotic disease caused by *Coxiella burnetii* that farmers can contract from their animals by inhalation
- **quarantine** the isolation of an individual for the purpose of preventing the spread of disease
- **quaternary ammonium salts (quats)** group of cationic detergents, named for the characteristic quaternary nitrogen atom that confers a positive charge, that make up an important class of disinfectants and antiseptics
- **quaternary structure** structure of protein complexes formed by the combination of several separate polypeptides or subunits

- **quinolines** class of antiprotozoan drugs long used for the treatment of malaria; interferes with heme detoxification
- **quorum sensing** cell-to-cell communication in bacteria; enables a coordinated response from cells when the population reaches a threshold density

R

- **R plasmid** plasmid containing genes encoding proteins that make a bacterial cell resistant to one or more antibiotics
- **rabies** contagious viral disease primarily transmitted by the bite of infected mammals that can cause acute encephalitis resulting in madness, aggressiveness, coma, and death
- **radial immunodiffusion** precipitin reaction in which antigen added to a well in an antiserum-impregnated gel diffuses, producing a precipitin ring whose diameter squared is directly proportional to antigen concentration
- **rat-bite fever** relapsing fever caused by either *Bacillus moniliformis* or *Spirillum minor*; can be transmitted by the bite of a rat or through contact with rat feces or urine
- **reaction center** protein complex in a photosystem, containing a pigment molecule that can undergo oxidation upon excitation by a light-harvesting pigment, actually giving up an electron
- **reactivation tuberculosis** secondary infection by *Mycobacterium tuberculosis* that forms later in life; occurs when the bacteria escape from the Ghon complexes and establish focal infections at other sites in immunocompromised individuals
- **reactive oxygen species (ROS)** unstable and toxic ions and molecules derived from partial reduction of oxygen
- **reading frame** way nucleotides in mRNA are grouped into codons
- **real-time PCR (quantitative PCR, qPCR)** a variant of PCR involving the use of fluorescence to allow for the monitoring of the increase in double-stranded template during a PCR reaction as it occurs, allowing for the quantitation of the original target sequence
- **receptor-mediated endocytosis** a type of endocytosis in which extracellular ligands are targeted to specific cells through their binding

to specific cell surface receptors

- **recognition site** a specific, often palindromic, DNA sequence recognized by a restriction enzyme that is typically four to six base pairs long and reads the same in the 5' to 3' direction on one strand as it does in the 5' to 3' direction on the complementary strand
- **recombinant DNA molecule** a DNA molecule resulting from the cutting and insertion of DNA from one organism into the DNA of another organism, resulting in a new combination of genetic material
- **recombinant DNA pharmaceuticals** pharmaceuticals produced as a result of genetic engineering
- **recombinant DNA technology** the process by which DNA from one organism is cut and new pieces of foreign DNA from a second organism are inserted, artificially creating new combinations of genetic material within the organism
- **redox potential** tendency for a molecule to acquire electrons and become reduced; electrons flow from molecules with lower redox potentials to those with higher redox potentials
- **redox reaction** pairing of an oxidation reaction with a reduction reaction
- **reduction reaction** chemical reaction that adds electrons to acceptor molecules, leaving them reduced
- **reemerging infectious disease** a disease that was once under control or largely eradicated that has begun causing new outbreaks due to changes in susceptible populations, the environment, or the pathogen itself
- **reflection** when light bounces back from a surface
- **refraction** bending of light waves, which occurs when a light wave passes from one medium to another
- **refractive index** a measure of the magnitude of slowing of light waves by a particular medium
- **regulatory T cells** class of T cells that are activated by self-antigens and serve to inhibit peripheral self-reacting T cells from causing damage and autoimmunity
- **rejection** process by which adaptive immune responses recognize transplanted tissue as non-self, mounting a response that destroys the tissue or leads to the death of the individual

- **relapsing fever** louse- or tickborne disease caused by *Borrelia recurrentis* or *B. hermsii* and characterized by a recurrent fever
- **replica plating** plating technique in which cells from colonies growing on a complete medium are inoculated onto various types of minimal media using a piece of sterile velvet, ensuring that the orientation of cells deposited on all plates is the same so that growth (or absence thereof) can be compared between plates
- **replication bubble** circular structure formed when the DNA strands are separated for replication
- **replication fork** Y-shaped structure that forms during the process of replication as DNA unwinds and opens up to separate the DNA strands
- **replication** process by which DNA is copied
- **reporter genes** genes that encode easily observable characteristics, allowing for their expression to be easily monitored
- **repressible operon** bacterial operon, that typically containing genes encoding enzymes required for a biosynthetic pathway and that is expressed when the product of the pathway continues to be required but is repressed when the product of the pathway accumulates, removing the need for continued expression
- **repressor** protein that suppresses transcription of a gene or operon in response to an external stimulus
- **reservoir** a living host or nonliving site in which a pathogenic organism can survive or multiply
- **resident microbiota** microorganisms that constantly live in the human body
- **resolution** the ability to distinguish between two points in an image
- **restriction endonuclease (restriction enzyme)** bacterial enzyme that cuts DNA fragments at a unique, often palindromic, recognition site; used in genetic engineering for splicing DNA fragments together into recombinant molecules
- **restriction fragment length polymorphism (RFLP)** a genetic variant identified by differing numbers or sizes of DNA fragments generated after digestion of a DNA sample with a restriction endonuclease; the variants are caused by the loss or gain of restriction sites, or the insertion or deleting of sequences between restriction sites.
- **retort** large industrial autoclave used for moist heat sterilization on a large scale

- **retrospective study** a research design that associates historical data with present cases
- **retrovirus** positive ssRNA virus that produces and uses reverse transcriptase to make an ssDNA copy of the retroviral genome that can then be made into dsDNA and integrate into the host cell chromosome to form a provirus within the host chromosome.
- **reverse transcriptase** enzyme found in retroviruses that can make a copy of ssDNA from ssRNA
- **reverse transcriptase inhibitor** classes of antiviral drugs that involve nucleoside analog competitive inhibition and non-nucleoside noncompetitive inhibition of the HIV reverse transcriptase
- **reverse transcriptase PCR (RT-PCR)** a variation of PCR used to obtain DNA copies of a specific mRNA molecule that begins with the conversion of mRNA molecules to cDNA by the enzyme reverse transcriptase
- **Reye syndrome** potentially life-threatening sequelae to some viral infections that result in the swelling of the liver and brain; aspirin use has also been linked to this syndrome
- **Rh factor** red blood cell surface antigen that can trigger type II hypersensitivity reactions
- **rheostat** a dimmer switch that controls the intensity of the illuminator on a light microscope
- **rheumatic fever** serious clinical sequela of an infection with *Streptococcus pyogenes* that can result in damage to joints or the valves of the heart
- **rheumatoid arthritis** systemic autoimmune disease in which immune complexes form and deposit in the joints and their linings, leading to inflammation and destruction
- **rhinitis** inflammation of the nasal cavity
- **rhizines** structures made of hyphae found on some lichens; aid in attachment to a surface
- **ribonucleic acid (RNA)** single-stranded nucleic acid composed of ribonucleotides; important in transcription and translation (protein synthesis)
- **ribonucleotides** RNA nucleotides containing ribose as the pentose sugar component and a nitrogenous base
- **ribosome** a complex intracellular structure that synthesizes proteins

- **riboswitch** small region of noncoding RNA found within the 5' end of some prokaryotic mRNA molecules that may bind to a small intracellular molecule, influencing the completion of transcription and/or translation
- **ribulose biphosphate carboxylase (RuBisCO)** first enzyme of the Calvin cycle responsible for adding a CO₂ molecule onto a five-carbon ribulose biphosphate (RuBP) molecule
- **rifampin** semisynthetic member of the rifamycin class that blocks bacterial RNA polymerase activity, inhibiting transcription
- **rimantadine** antiviral drug that targets the influenza virus by preventing viral escape from endosomes upon host cell uptake, preventing viral RNA release and subsequent viral replication
- **ringworm** a tinea (cutaneous mycosis of the skin), typically characterized by a round, red, slightly raised lesion that heals outward from the center, giving it the appearance of a round worm
- **RNA interference (RNAi)** process by which antisense RNAs or small interfering RNAs (siRNAs) interfere with gene expression by binding to mRNA, preventing translation and protein synthesis
- **RNA polymerase** enzyme that adds nucleotides to the 3'-OH group of the growing mRNA molecule that are complementary to the template strand, forming covalent phosphodiester bonds between the nucleotides in the RNA
- **RNA splicing** process of removing intron-encoded RNA sequences from eukaryotic primary transcripts and reconnecting those encoded by exons
- **RNA transcript** mRNA produced during transcription
- **Rocky Mountain spotted fever** potentially fatal tickborne disease caused by *Rickettsia rickettsii* characterized by fever, body aches, and a rash
- **rogue form** misfolded form of the PrP protein that is normally found in the cell membrane and has the tendency to aggregate in neurons, causing extensive cell death and brain damage
- **rolling circle replication** type of rapid unidirectional DNA synthesis of a circular DNA molecule
- **roseola** a rash-causing illness, most commonly affecting children, associated with human herpesvirus 6 (HHV-6)

- **rough endoplasmic reticulum** a type of endoplasmic reticulum containing bound 80S ribosomes for the synthesis of proteins destined for the plasma membrane
- **route of administration** method used to introduce a drug into the body
- **rRNA** type of stable RNA that is a major constituent of ribosomes, ensuring proper alignment of the mRNA and the ribosomes as well as catalyzing the formation of the peptide bonds between two aligned amino acids during protein synthesis
- **rubella** German measles, caused by the rubella virus
- **runs (running)** purposeful, directional movement of a prokaryotic cell propelled by counterclockwise flagellar rotation

S

- **σ factor** subunit of bacterial RNA polymerase conferring promoter specificity that can be substituted with a different version in response to an environmental condition, allowing for a quick and global change of the regulon transcribed
- **saccharide** carbohydrate
- **salmonellosis** gastrointestinal illness caused by *Salmonella* bacteria
- **salpingitis** inflammation of the fallopian tubes
- **sandwich ELISA** EIA in which the primary antibody is first attached to the wells of a microtiter plate, allowing it to capture antigen from an unknown solution to be quantified
- **Sanger DNA sequencing (dideoxy method, chain termination method)** the original DNA sequencing technique in which dideoxy nucleotides, each labeled with a molecular beacon, are used to terminate chain elongation; the resulting incrementally sized fragments are then separated by electrophoresis to determine the sequence of the DNA molecule
- **sanitization** protocol that reduces microbial load on inanimate surfaces to levels deemed safe for public health
- **saprophytic** refers to protozoans that ingest small, soluble food molecules

- **SARS** severe acute respiratory syndrome; caused by a zoonotic coronavirus that results in flu-like symptoms
- **saturated fatty acid** lipid with hydrocarbon chains containing only single bonds, which results in the maximum number of hydrogen atoms per chain
- **scanning electron microscope (SEM)** a type of electron microscope that bounces electrons off of the specimen, forming an image of the surface
- **scanning probe microscope** a microscope that uses a probe that travels across the surface of a specimen at a constant distance while the current, which is sensitive to the size of the gap, is measured
- **scanning tunneling microscope** a microscope that uses a probe that is passed just above the specimen as a constant voltage bias creates the potential for an electric current between the probe and the specimen
- **scarlet fever** bacterial infection caused by *Streptococcus pyogenes*, marked by a high fever and a disseminated scarlet rash
- **schistosomiasis** helminthic infection caused by *Schistosoma* spp.; transmitted from a snail intermediate host to human swimmers or bathers in freshwater
- **schizogony** asexual reproduction in protozoans that is characterized by multiple cell divisions (one cell dividing to form many smaller cells)
- **scolex** the head region of a cestode (tapeworm), which typically has suckers and/or hooks for attachment to the host
- **scrapie** form of transmissible spongiform encephalopathy that primarily affects sheep
- **sebaceous gland** a gland located in hair follicles that secretes sebum
- **sebum** lipid-rich substance secreted by the sebaceous glands of the skin
- **secondary antibody** antibody to which an enzyme is attached for use in ELISA assays; in direct and sandwich ELISAs, it is specific for the antigen being quantified, whereas in indirect ELISA, it is specific for the primary antibody
- **secondary immunodeficiency** impaired immune response due to infection, metabolic disturbance, poor diet, stress, or other acquired factors
- **secondary infection** second infection that develops after a primary infection as a result of the primary disease compromising immune

- defenses or antibiotics, thus eliminating protective microbiota
- **secondary lymphoid tissue** one of two types of lymphatic tissue; comprises the spleen, lymph nodes, Peyer's patches, and mucosa associated lymphoid tissue (MALT)
 - **secondary response** the adaptive immune response produced in response to a specific antigen to which the body has previously been exposed
 - **secondary structure** structure stabilized by hydrogen bonds between the carbonyl and amine groups of a polypeptide chain; may be an α -helix or a β -pleated sheet, or both
 - **secretory vesicle** membranous sac that carries molecules through the plasma membrane to be released (secreted) from the cell
 - **selective IgA deficiency** primary immunodeficiency in which individuals produce normal levels of IgG and IgM, but are unable to produce secretory IgA
 - **selective media** media that contain additives that encourage the growth of some bacteria while inhibiting others
 - **selective toxicity** desirable quality of an antimicrobial drug indicating that it preferentially kills or inhibits the growth of the target microbe while causing minimal or no harm to the host
 - **semiconservative DNA replication** pattern of DNA replication process whereby each of the two parental DNA strands acts as a template for new DNA to be synthesized, producing hybrid old- and new-strand daughter molecules
 - **semicritical item** object that contacts mucous membranes or nonintact skin but does not penetrate tissues; requires a high level of disinfection
 - **seminal vesicles** glands that contribute fluid to semen
 - **semisynthetic antimicrobial** chemically modified derivative of a natural antibiotic
 - **sense strand** strand of DNA that is not transcribed for gene expression; it is complementary to the antisense strand
 - **sepsis** systemic inflammatory response to an infection that results in high fever and edema, causing organ damage and possibly leading to shock and death
 - **septate hyphae** hyphae that contain walls between individual cells; characteristic of some fungi
 - **septic arthritis** see *infectious arthritis*

- **septic shock** serious condition marked by the loss of blood pressure resulting from an inflammatory response against a systemic infection
- **septic** the condition of being septicemic; having an infection in the blood
- **septicemia** condition in which pathogens are multiplying in blood
- **septicemic plague** form of plague that occurs when the bacterial pathogen gains access to the bloodstream
- **septum** separating structure that forms during cell division; also describes the separating wall between cells in a filament
- **sequela (plural: sequelae)** condition that arises as a consequence of a prior disease
- **serial dilution** sequential transfer of known volumes of culture samples from one tube to another to perform a several-fold dilution of the original culture
- **seroconversion** point in an infection at which antibody to a pathogen is detectible using an immunoassay
- **serotype** strain or variation of the same species of bacteria; also called serovar
- **serovar** specific strain of bacteria identified by agglutination using strain-specific antisera
- **serum** fluid portion of the blood after clotting has occurred; generally lacks clotting factors
- **serum sickness** systemic type III hypersensitivity reaction
- **sessile** attached to a surface
- **severe combined immunodeficiency disease (SCID)** genetic disorder resulting in impaired function of B cells and T cells
- **sex pilus** specialized type of pilus that aids in DNA transfer between some prokaryotic cells
- **sheath** part of the tail on a bacteriophage that contracts to introduce the viral DNA into the bacterium
- **shigellosis** gastrointestinal illness caused by *Shigella* bacteria, also called bacillary dysentery
- **shingles** acute and painful rash that forms following the reactivation of a latent chickenpox infection
- **shock** extreme drop in blood pressure that, among other causes, can result from a strong immune response to the activity of toxins or response to bacterial products and can result in death

- **shuttle vector** a plasmid that can move between bacterial and eukaryotic cells
- **side chain** the variable functional group, R , attached to the α carbon of an amino acid
- **sign** objective and measurable indication of a disease
- **silent mutation** point mutation that results in the same amino acid being incorporated into the resulting polypeptide
- **simple microscope** a type of microscope with only one lens to focus light from the specimen
- **simple staining** a staining technique that uses a single dye
- **single-stranded binding protein** protein that coats the single strands of DNA near each replication fork to prevent the single-stranded DNA from rewinding into a double helix
- **sinusitis** inflammation of the sinuses
- **S-layer** cell envelope layer composed of protein covering the cell walls of some bacteria and archaea; in some archaea, may function as the cell wall
- **slime layer** a type of glycocalyx with unorganized layers of polysaccharides that aid bacterial adherence to surfaces
- **smear** a thin layer of a specimen on a slide
- **smooth endoplasmic reticulum** a type of endoplasmic reticulum that lacks ribosomes, is involved in the biosynthesis of lipids and in carbohydrate metabolism, and serves as the site of detoxification of toxic compounds within the cell
- **soft chancres** soft, painful ulcers associated with the STI chancroid
- **soma** cell body of a neuron
- **sonication** method of microbial control that involves application of ultrasound waves to form cavitation within a solution, including inside cells, disrupting cell components as a result
- **Southern blot** a technique in molecular genetics used to detect the presence of certain DNA sequences within a given DNA sample; DNA fragments within the sample are separated by agarose gel electrophoresis, immobilized on a membrane, and then exposed to a specific DNA probe labeled with a radioactive or fluorescent molecular beacon to aid in detection
- **specialized transduction** transfer of a specific piece of bacterial chromosomal DNA near the site of integration by the phage

- **specificity** the ability of the specific adaptive immune system to target specific pathogens or toxins
- **spike** viral glycoprotein embedded within the viral capsid or envelope used for attachment to host cells
- **spirochetes** a group of long, thin, spiral-shaped fastidious bacteria that includes the human pathogens that cause syphilis, Lyme disease, and leptospirosis
- **spleen** abdominal organ consisting of secondary lymphoid tissue that filters blood and captures pathogens and antigens that pass into it; also contains specialized macrophages and dendritic cells that are crucial for antigen presentation
- **spliceosome** protein complex containing small nuclear ribonucleoproteins that catalyzes the splicing out of intron-encoded RNA sequences from the primary transcript during RNA maturation in eukaryotes
- **spontaneous generation** the now-disproven theory that life can arise from nonliving matter
- **spontaneous mutation** mutation not caused by a mutagen that occurs through DNA replication errors
- **sporadic disease** an illness that occurs at relatively low levels with no discernible pattern or trend, frequently with no geographic focus
- **spores** specialized cells that may be used for reproduction or may be specialized to withstand harsh conditions
- **sporotrichosis** subcutaneous infection caused by the fungus *Sporothrix schenckii*, which causes skin lesions and can potentially spread to the lymphatic system; also known as rose gardener's disease or rose thorn disease
- **sporulation** the process by which a vegetative cell produces a dormant endospore
- **spread plate method** a technique used for inoculating plates with diluted bacterial samples for the purpose of cell counting; the liquid sample is pipetted onto solid medium and spread uniformly across the plate
- **St. Louis encephalitis** mosquito-borne viral infection of the brain that occurs primarily in the central and southern United States
- **stage** the platform of a microscope on which slides are placed

- **staining** the addition of stains or dyes to a microscopic specimen for the purpose of enhancing contrast
- **staphylococcal food poisoning** gastrointestinal illness caused by toxins produced by *Staphylococcus aureus*
- **staphylolysins** a class of staphylococcal exotoxins that are cytotoxic to skin cells and white blood cells
- **starch** energy-storage polysaccharide in plants; composed of two types of glucose polymers: amylose and amylopectin
- **start codon** AUG codon, specifying methionine, which is typically the codon that initiates translation
- **stationary phase** interval during which the number of cells formed by cell division is equal to the number of cells dying
- **stereoisomers** isomers that differ in the spatial arrangements of atoms
- **sterilant** strong chemical that effectively kills all microbes and viruses in or on an inanimate item
- **sterile field** specified area that is free of all vegetative microbes, endospores, and viruses
- **sterilization** protocol that completely removes all vegetative cells, endospores, and viruses from an item
- **steroid** lipid with complex, ringed structures found in cell membranes and hormones
- **sterol** the most common type of steroid; contains an OH group at one specific position on one of the molecule's carbon rings
- **sticky ends** short, single-stranded complementary overhangs that may be produced when many restriction enzymes cut DNA
- **stigma** light-sensing eyespot found in *Euglena*
- **stop codon (nonsense codon)** one of three codons for which there is no tRNA with a complementary anticodon; a signal within the mRNA for termination of translation
- **stratum corneum** a layer of dead, keratinized cells that forms the uppermost layer of the epidermis
- **strep throat (streptococcal pharyngitis)** bacterial pharyngitis caused by *Streptococcus pyogenes*
- **streptococcal toxic shock-like syndrome (STSS)** condition similar to staphylococcal toxic shock syndrome but with greater likelihood of bacteremia, necrotizing fasciitis, and acute respiratory distress syndrome

- **stroma** a gel-like fluid that makes up much of a chloroplast's volume, and in which the thylakoids float
- **strongyloidiasis** soil-transmitted intestinal infection caused by the helminth *Strongyloides stercoralis*
- **structural formula** graphic representation of the molecular structure showing how the atoms are arranged
- **structural isomers** molecules composed of the same numbers and types of atoms but with different bonding sequences
- **subacute bacterial endocarditis** form of endocarditis in which damage to the valves of the heart occurs over months as a result of blood clot formation and immune-response-induced fibrosis of the valves
- **subclinical disease** disease that does not present any signs or symptoms
- **subcutaneous mycosis** any fungal infection that penetrates the epidermis and dermis to enter deeper tissues
- **substrate** chemical reactants of an enzymatic reaction
- **substrate-level phosphorylation** direct method of ATP production in which a high-energy phosphate group is removed from an organic molecule and added to an ADP molecule
- **subunit vaccine** vaccine that contains only key antigens as opposed to whole pathogens
- **sugar-phosphate backbone** alternating sugar-phosphate structure composing the framework of a nucleic acid strand that results from phosphodiester bond formation between nucleotides
- **sulfonamides (sulfa drugs)** group of structurally related synthetic antimicrobial compounds that function as antimetabolites, competitively inhibiting an enzyme in the bacterial folic acid synthesis pathway
- **superantigen** class of exotoxin that triggers a strong nonspecific immune response with excessive production of cytokines (cytokine storm) causing inflammation, high fever, shock, and, potentially, death
- **supercoiled** extensive wrapping and twisting of a DNA molecule, allowing the DNA to fit within a small space
- **supercoiling** process in which DNA is underwound or overwound to fit inside a cell

- **supercritical fluid** molecule, commonly carbon dioxide, brought to high pressures to reach a state that has physical properties between those of liquids and gases, allowing it to effectively penetrate surfaces and cells to form carbonic acid, which lowers the pH of cells considerably, leading to sterilization
- **superinfection** secondary infection that may develop as a result of long-term, broad-spectrum antimicrobial use
- **superoxide dismutase** enzyme that catalyzes the breakdown of superoxide anions
- **suppurative** producing pus; purulent
- **surfactant** group of chemical compounds used for degerming; lower the surface tension of water, creating emulsions that mechanically carry away microorganisms
- **sweat gland** one of numerous tubular glands embedded in the dermis that secretes the watery substance known as perspiration
- **symbiosis** any interaction between different species that are associated with each other within a community
- **symptom** subjective experience of disease felt by the patient
- **synapse** junction between a neuron and another cell
- **syncytia** multinucleated cells that form from the fusion of normal cells during infections or other processes
- **syndrome** group of signs and symptoms characteristic of a particular disease
- **syngamy** process in which haploid gametes fuse
- **synthetic antimicrobial** antimicrobial developed from a chemical not found in nature
- **syphilis** an STI caused by the bacterium *Treponema pallidum*
- **systemic autoimmune disease** autoimmune disease that affect the organism as a whole, rather than a single organ
- **systemic infection** infection that has spread to multiple locations or body systems
- **systemic inflammatory response syndrome (SIRS)** severe inflammatory response to the presence of microbes in the blood; can lead to sepsis
- **systemic lupus erythematosus (SLE)** systemic autoimmune disease producing inflammatory type III hypersensitivities as antibodies form immune complexes with nuclear and cytoplasmic antigens

- **systemic mycosis** a fungal infection that spreads throughout the body

T

- **T-cell receptors (TCR)** molecules on T cells involved in the recognition of processed foreign epitopes presented with MHC I or MHC II
- **T lymphocyte** lymphocyte that serves as the central orchestrator, bridging humoral, cellular, and innate immunity, and serves as the effector cells of cellular immunity; T cell
- **taeniasis** infection caused by *Taenia* or *Diphyllbothrium*
- **tail fiber** long protein component on the lower part of a phage used for specific attachment to bacterial cell
- **tail pins** points extended at the base of a bacteriophage sheath that, along with tail fibers, lead to phage attachment to a bacterial cell
- **tapeworms** segmented, hermaphroditic, parasitic flatworms (Platyhelminthes)
- **tartar** calcified heavy plaque on teeth, also called dental calculus
- **taxonomy** the classification, description, identification, and naming of living organisms
- **T-dependent antigen** a protein antigen that is only capable of activating a B cell with the cooperation of a helper T cell
- **TDP** thermal death point is the lowest temperature at which all microorganisms are killed in a 10-minute exposure
- **TDT** thermal death time is the length of time needed to kill all microorganisms in a sample at a given temperature
- **telomerase** enzyme that attaches to the end of a linear chromosome and adds nucleotides to the 3' end of one of the DNA strands, maintaining the telomere sequence, thus preventing loss of DNA from the end of the chromosome
- **telomere** repetitive, noncoding sequence found at the end of a linear eukaryotic chromosome that protects the genes near the end of the chromosome from deletion as the DNA molecule is repeatedly replicated
- **temperate phage** bacteriophage that can incorporate viral genome into the host cell chromosome and replicate with the host cell until new

- viruses are produced; a phage that undergoes the lysogenic cycle
- **teratogenic** able to disrupt the normal development of a fetus in utero
 - **terbinafine** antifungal drug of the allylamine class that is used topically for the treatment of dermatophytic skin infections
 - **termination of DNA replication** stage of replication during which DNA replication is halted once the chromosome has been fully replicated
 - **termination of transcription** stage of transcription that occurs when RNA polymerase has reached specific DNA sequences, leading to release of the enzyme from the DNA template, freeing the RNA transcript and, thus, halting transcription
 - **termination of translation** stage of translation during which a nonsense codon aligns with the A site, signaling release factors to release of the polypeptide, leading to the dissociation of the small and large ribosomal subunits from the mRNA and from each other
 - **tertiary structure** large-scale, three-dimensional structure of a polypeptide
 - **test sensitivity** probability that a diagnostic test will find evidence of the targeted disease when the pathogen is present
 - **test specificity** probability that a diagnostic test will not find evidence of the targeted disease when the pathogen is absent
 - **testes** (singular *testis*) pair of glands located in the scrotum of males that produce sperm and testosterone
 - **tetanus** bacterial disease caused by exotoxin produced by *Clostridium tetani* that causes a rigid paralysis
 - **tetracyclines** class of protein synthesis inhibitors that bind to the 30S subunit, blocking the association of tRNAs with the ribosome during translation
 - **T_H1 cells** subtype of T cells that stimulate cytotoxic T cells, macrophages, neutrophils, and NK cells
 - **T_H17 cells** subtype of T cell that are essential for defense against specific pathogens and infections, such as chronic mucocutaneous infections with *C. albicans*
 - **T_H2 cells** subtype of T cells that stimulate B cells and direct their differentiation; also involved in directing antibody class switching
 - **thallus** body of fleshy fungi (more generally, a body without a root, stem, or leaf) that commonly co-occurs with HIV infection; the

microbes move to the lymphatic system in the groin

- **thermophile** a microorganism that grows best at warm temperatures, typically between about 50 °C and 80 °C
- **thin sections** thin slices of tissue for examination under a TEM
- **thioglycolate medium** medium designed to test the aerotolerance of bacteria; it contains a low concentration of agar to allow motile bacteria to move throughout the medium
- **thioglycolate tube culture** contains reducing medium through which oxygen diffuses from the tube opening, producing a range of oxygen environments down the length of the tube
- **thrombocytes** see *platelets*
- **thylakoids** a highly dynamic collection of membranous sacs found in the stroma of chloroplasts; site of photosynthesis
- **thymic selection** a three-step process of negative and positive selection of T cells in the thymus
- **thymine dimer** covalent linkage between two adjacent thymine bases on exposure to ultraviolet radiation
- **thymine** pyrimidine nitrogenous base found only in DNA nucleotides
- **tincture** solution of an antiseptic compound dissolved in alcohol
- **T-independent antigen** a nonprotein antigen that can activate a B cell without cooperation from a helper T cell
- **tinea** any cutaneous fungal infection caused by dermatophytes, such as tinea corporis, tinea capitis, tinea cruris, and tinea pedis
- **tinea capitis** cutaneous mycosis of the scalp; also known as ringworm of the scalp
- **tinea corporis** cutaneous mycosis of the body; also known as ringworm of the body
- **tinea cruris** cutaneous mycosis of the groin region; also known as jock itch
- **tinea pedis** cutaneous mycosis of the feet; also known as athlete's foot
- **tissue tropism** tendency of most viruses to infect only certain tissue types within a host
- **titer** concentration obtained by titration; the reciprocal of a measurement of biological activity determined by finding the dilution of an unknown (e.g., antigen-specific antibody in an antiserum) that shows the defined end-point; always expressed as a whole number
- **tolerance** lack of an anti-self immune response

- **toll-like receptors (TLRs)** pathogen recognition receptors (PRRs) that may be found on the external surface of phagocytes or facing inward in interior compartments
- **tonsillitis** inflammation of the tonsils
- **topoisomerase** type of enzyme that helps maintain the structure of supercoiled chromosomes, preventing overwinding of DNA during certain cellular processes like DNA replication
- **topoisomerase II** enzyme responsible for facilitating topological transitions of DNA, relaxing it from its supercoiled state
- **total magnification** in a light microscope is a value calculated by multiplying the magnification of the ocular by the magnification of the objective lenses
- **toxemia** presence of toxins in the blood
- **toxic shock syndrome** severe condition marked by the loss of blood pressure and blood clot formation caused by a bacterial superantigen, toxic shock syndrome toxin
- **toxigenicity** ability of a pathogen to produce toxins to cause damage to host cells
- **toxin** poison produced by a pathogen
- **toxoid vaccine** vaccine that contains inactivated bacterial toxins
- **toxoplasmosis** typically asymptomatic protozoan infection caused by *Toxoplasma* spp. and transmitted through contact with cysts in cat feces; infections in pregnant women may cause birth defects or miscarriage
- **trace element** indispensable element present in cells in lower amounts than macronutrients; also called *micronutrient*
- **trachea** also known as the windpipe, this is a stiffened tube of cartilage that runs from the larynx to the bronchi
- **trachoma** a type of conjunctivitis, caused by *Chlamydia trachomatis*, that is a major cause of preventable blindness
- **transcription bubble** region of unwinding of the DNA double helix during transcription
- **transcription factors** proteins encoded by regulatory genes that function by influencing the binding of RNA polymerase to the promoter and allowing its progression to transcribe structural genes
- **transcription** process of synthesizing RNA using the information encoded in DNA

- **transcriptomics** the study of the entire collection of mRNA molecules produced by cells; involves monitoring differences in gene expression patterns between cells at the mRNA level
- **transduction** mechanism of horizontal gene transfer in bacteria in which genes are transferred through viral infection
- **transendothelial migration** process by which circulating leukocytes exit the bloodstream via the microvascular endothelium
- **transfection** the introduction of recombinant DNA molecules into eukaryotic hosts
- **transformation** mechanism of horizontal gene transfer in bacteria in which naked environmental DNA is taken up by a bacterial cell
- **transgenic** describing an organism into which foreign DNA from a different species has been introduced
- **transient microbiota** microorganisms, sometimes pathogenic, that are only temporarily found in the human body
- **transition reaction** reaction linking glycolysis to the Krebs cycle, during which each pyruvate is decarboxylated and oxidized (forming NADH), and the resulting two-carbon acetyl group is attached to a large carrier molecule called coenzyme A, resulting in the formation of acetyl-CoA and CO₂; also called the *bridge reaction*
- **translation (protein synthesis)** process of protein synthesis whereby a ribosome decodes an mRNA message into a polypeptide product
- **transmissible spongiform encephalopathy** degenerative disease caused by prions; leads to the death of neurons in the brain
- **transmission electron microscope (TEM)** a type of electron microscope that uses an electron beam, focused with magnets, that passes through a thin specimen
- **transmittance** the amount of light that passes through a medium
- **transparency** the property of allowing light to pass through
- **transport vesicle** membranous sac that carries molecules between various components of the endomembrane system
- **transposition** process whereby a DNA sequence known as a transposon independently excises from one location in a DNA molecule and integrates elsewhere
- **transposon (transposable element)** molecule of DNA that can independently excise from one location in a DNA molecule and integrate into the DNA elsewhere

- **trench fever** louseborne disease caused by *Bartonella quintana* and characterized by high fever, body aches, conjunctivitis, ocular pain, severe headaches, and severe bone pain
- **trench mouth** a severe form of gingivitis, also called acute necrotizing ulcerative gingivitis
- **treponemal serologic tests** tests for syphilis that measure the amount of antibody directed against antigens associated with *Treponema pallidum*
- **triacylglycerol** three fatty acids chemically linked to a glycerol molecule; also called a triglyceride
- **triazoles** ergosterol biosynthesis inhibitors used to treat several types of systemic yeast infections; exhibit more selective toxicity than the imidazoles and are associated with fewer side effects
- **tricarboxylic acid cycle** see *Krebs cycle*
- **trichinosis** soil-transmitted intestinal infection caused by the nematode *Trichinella spiralis*; associated with cyst formation
- **trichomoniasis** a common STI caused by *Trichomonas vaginalis*
- **trichuriasis** intestinal infection caused by the whipworm *Trichuris trichiura*
- **triglyceride** three fatty acids chemically linked to a glycerol molecule; also called a triacylglycerol
- **trimethoprim** synthetic antimicrobial compound that functions as an antimetabolite to an enzyme in the bacterial folic acid synthesis pathway
- **tRNA** small type of stable RNA that carries the correct amino acid to the site of protein synthesis in the ribosome and base pairs with the mRNA to allow the amino acid it carries to be inserted in the polypeptide chain being synthesized
- **trophozoite** a life cycle phase in which protists are actively feeding and growing
- **tubercle** small, rounded lesion
- **tuberculosis** life-threatening form of microbial infection marked by the presence of acid-fast bacteria growing in nodules (especially in the lungs)
- **tularemia** infection of the lymphatic system by *Francisella tularensis*; also known as rabbit fever

- **tumbles (tumbling)** random, circuitous movement of a bacterial cell, propelled by clockwise flagellar rotation
- **tumor** collection or aggregate of cells; can be benign (noncancerous) or malignant (cancerous)
- **tumor-inducing (Ti) plasmid** a naturally occurring plasmid of the bacterium *Agrobacterium tumefaciens* that researchers use as a shuttle vector to introduce a desired DNA fragment into plant cells
- **turbidity** cloudiness of a culture due to refraction of light by cells and particles
- **two-photon microscope** a microscope that uses long-wavelength or infrared light to fluoresce fluorochromes in the specimen
- **tympanic membrane** also referred to as the ear drum, this structure separates the outer and middle ear
- **type 1 diabetes mellitus** hyperglycemia caused by an autoimmune disease affecting insulin production by β cells of the pancreas
- **type I hypersensitivity** rapid-onset allergic reaction due to cross-linking of antigen-specific IgE on the outside of mast cells, resulting in release of inflammatory mediators
- **type II hypersensitivity** cytotoxic reaction triggered by IgG and IgM antibodies binding to antigens on cell surfaces
- **type III hypersensitivity** inflammatory reaction induced by formation of immune complexes and their deposition in tissues and blood vessels
- **type IV hypersensitivity** delayed T-cell-mediated inflammatory reaction that takes longer to manifest than the first three hypersensitivity types, due to the need for activation of antigen-presenting cell and T-cell subsets
- **typhoid fever** serious illness caused by infection with certain serotypes of *Salmonella*

U

- **UHT pasteurization** method of pasteurization that exposes milk to ultra-high temperatures (near 140 °C) for a few seconds, effectively sterilizing it so that it can be sealed and stored for long periods without refrigeration
- **ulcer** open sore

- **ultramicrotome** a device that cuts thin sections for electron microscopy
- **unit membrane** biological membrane composed of two layers of phospholipid molecules with the nonpolar tails associating to form a hydrophobic barrier between the polar heads; also called lipid bilayer
- **unsaturated fatty acid** lipid with hydrocarbon chains containing one or more carbon-carbon double bonds and subsequently fewer than the maximum number of hydrogen atoms per chain
- **uracil** pyrimidine nitrogenous base found only in RNA nucleotides
- **ureter** duct that transports urine from the kidneys to the urinary bladder
- **ureteritis** inflammation of the ureter
- **urethra** duct through which urine passes from the urinary bladder to leave the body through the urinary meatus
- **urethritis** inflammation of the urethra
- **urinary bladder** an organ that stores urine until it is ready to be excreted
- **urinary meatus** the opening through which urine leaves the body
- **use-dilution test** a technique for determining the effectiveness of a chemical disinfectant on a surface; involves dipping a surface in a culture of the targeted microorganism, disinfecting the surface, and then transferring the surface to a fresh medium to see if bacteria will grow
- **uterus** female reproductive organ in which a fertilized egg implants and develops

V

- **vaccination** inoculation of a patient with attenuated pathogens or antigens to activate adaptive immunity and protect against infection
- **vagina** female reproductive organ that extends from the vulva to the cervix
- **vaginitis** inflammation of the vagina
- **vaginosis** an infection of the vagina caused by overgrowth of resident bacteria
- **vancomycin** cell wall synthesis inhibitor of the glycopeptide class

- **vancomycin-intermediate *Staphylococcus aureus* (VISA)** pathogen with intermediate vancomycin resistance due to increased targets for and trapping of vancomycin in the outer cell wall
- **vancomycin-resistant enterococci (VRE)** pathogens resistant to vancomycin through a target modification of peptidoglycan subunit peptides that inhibit binding by vancomycin
- **vancomycin-resistant *Staphylococcus aureus* (VRSA)** pathogen with resistance to vancomycin that has arisen as a result of the horizontal gene transfer of vancomycin resistance genes from VRE
- **variolation** the historical practice of inoculating a healthy patient with infectious material from a person infected with smallpox in order to promote immunity to the disease
- **vas deferens** pair of ducts in the male reproductive system that conduct sperm from the testes and seminal fluid to the ejaculatory duct
- **vasculitis** inflammation affecting blood vessels (either arteries or veins)
- **VDRL (Venereal Disease Research Laboratory) test** test for syphilis that detects anti-treponemal antibodies to the phospholipids produced due to the tissue destruction by *Treponema pallidum*; antibodies are detected through a flocculation reaction with cardiolipin extracted from beef heart tissue
- **vector** animal (typically an arthropod) that transmits a pathogen from one host to another host; DNA molecules that carry DNA fragments from one organism to another
- **vegetative cell** a cell that is actively growing and dividing, and does not contain an endospore
- **vehicle transmission** transfer of a pathogen between hosts via contaminated food, water, or air
- **vein** blood vessel that returns blood from the tissues to the heart for recirculation
- **vertical direct transmission** transfer of a pathogen from mother to child during pregnancy, birth, or breastfeeding
- **vertical gene transfer** transfer of genes from parent to offspring
- **viable cell** live cell; live cells are usually detected as colony-forming units
- **viable plate count** direct method of measuring microbial growth in a culture; the number of viable or live cells is usually expressed in

CFU/mL

- **viral conjunctivitis** inflammation of the conjunctiva caused by a viral infection
- **viral envelope** lipid membrane obtained from phospholipid membranes of the cell that surrounds the capsid
- **viral hemagglutination inhibition assay** assay used to quantify the amount of neutralizing antibody against a virus by showing a decrease in hemagglutination caused by a standardized amount of virus
- **viral titer** number of virions per unit volume
- **viremia** presence of virus in blood
- **viricide** chemical or physical treatment that destroys or inactivates viruses
- **virion** inert particle that is the reproductive form of a virus
- **viroid** infectious plant pathogen composed of RNA
- **virology** the study of viruses
- **virulence** degree to which an organism is pathogenic; severity of disease signs and symptoms
- **virulence factor** product of a pathogen that assists in its ability to cause infection and disease
- **virulent phage** bacteriophage for which infection leads to the death of the host cell; a phage that undergoes the lytic cycle
- **virus** an acellular microorganism, consisting of proteins and genetic material (DNA or RNA), that can replicate itself by infecting a host cell
- **virusoid** small piece of RNA associated with larger RNA of some infectious plant viruses
- **volutin** inclusions of polymerized inorganic phosphate; also called metachromatic granules
- **vulva** the female external genitalia

W

- **water activity** water content of foods or other materials
- **wavelength** the distance between one peak of a wave and the next peak

- **Weil's disease** advanced stage of leptospirosis in which the kidney and liver become seriously infected
- **West African trypanosomiasis** chronic form of African trypanosomiasis caused by *Trypanosoma brucei gambiense*
- **West Nile encephalitis** mosquito-borne disease caused by the West Nile virus (WNV) that can result in swelling of the brain and death in severe cases
- **western blot** technique used to detect the presence of a certain protein within a given protein sample in which proteins within the sample are separated by PAGE, immobilized on a membrane, and then exposed first to an antibody that binds to the protein of interest and then second to an antibody equipped with a molecular beacon that will bind to the first antibody
- **western equine encephalitis** serious but rare mosquito-borne viral infection of the brain that is found primarily in the central and western United States
- **wet mount** a slide preparation technique in which a specimen is placed on the slide in a drop of liquid
- **wheal-flare reaction** localized type I hypersensitivity reaction, involving a raised, itchy bump (wheal) and redness (flare), to injected allergen
- **whooping cough** common name for pertussis
- **wild type** phenotype of an organism that is most commonly observed in nature
- **Winterbottom's sign** acute swelling of lymph nodes at the back of the neck that is an early sign of African trypanosomiasis
- **wobble position** third position of a codon that, when changed, typically results in the incorporation of the same amino acid because of the degeneracy of the genetic code
- **World Health Organization (WHO)** international public health organization within the United Nations; monitors and communicates international public health information and coordinates international public health programs and emergency interventions

- **xenobiotic** compound synthesized by humans and introduced to an environment in much higher concentrations than expected in nature
- **xenograft** transplanted tissue from a donor that is of a different species than the recipient
- **X-linked agammaglobulinemia** genetic disorder resulting in an inability to produce antibodies
- **x-y mechanical stage knobs** knobs on a microscope that are used to adjust the position of the specimen on the stage surface, generally to center it directly above the light

Y

- **yeast** any unicellular fungus
- **yeast infection** fungal infection of the vagina typically caused by an overgrowth of resident *Candida* spp.
- **yellow fever** mild to potentially fatal mosquito-borne viral disease caused by the yellow fever virus

Z

- **Ziehl-Neelsen technique** a method of acid-fast staining that uses heat to infuse the primary stain, carbolfuchsin, into acid-fast cells
- **zone of inhibition** clear zone around a filter disk impregnated with an antimicrobial drug, indicating growth inhibition due to the antimicrobial drug
- **zoonosis** see *zoonotic disease*
- **zoonotic disease** any disease that is transmitted to humans by animals
- **zooplankton** heterotrophic plankton
- **Z-scheme** electron flow seen in noncyclic photophosphorylation in plants, algae, and cyanobacteria due to the use of both PSI and PSII
- **zygospores** spores used by Zygomycetes for sexual reproduction; they have hard walls formed from the fusion of reproductive cells from two individuals